American Academy of Allergy, Twelfth Annual Meeting, Chase–Park Plaza Hotels, St. Louis, Missouri, February 6, 7, 8, 1956

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The Journal of Allergy

VOL. 26 SEPTEMBER, 1955 No. 5

Original Articles

CORRELATION OF BLOOD HISTAMINE RELEASE AND SKIN TEST RESPONSE TO MULTIPLE ANTIGENS

JOSEPH W. NOAH, M.D., AND ALTA BRAND, ST. LOUIS, MO.

IT HAS been shown previously that histamine is transferred from the cells to the plasma when blood from a ragweed-sensitive person is incubated with this antigen in vitro.[1, 2] The present study reports the results obtained when the same technique was employed with other inhalant and food antigens in order to determine (a) whether the release of histamine from cells could be obtained with such antigens; (b) whether a correlation could be demonstrated between histamine release and the skin response to intracutaneous injection of the given antigen; and (c) the accuracy of histamine release as a means of defining the substance responsible for allergic manifestations in those situations where no parallelism between skin response and histamine release could be demonstrated.

METHODS

Selection of Patients.—Included in this study were blood specimens of fourteen patients who were chosen because they presented a clinical syndrome easily identifiable as allergic in origin, usually asthma or hayfever, or both. In some cases the allergens could be identified by history and substantiated by skin test results, but in other patients they had not been identified even after long periods of observation.

Blood for control determinations was obtained from four donors who had had, at the most, brief and isolated instances of allergic illness.

Procedure.—A 100 ml. sample of whole blood was obtained from the antecubital vein of the donors on each of three visits to the laboratory. In a few persons, 150 ml. was obtained at a sitting, so that only two visits were required. All blood samples were collected in syringes wet with a solution containing 10 mg.

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of sodium heparin per milliliter. A total leukocyte count was performed upon each sample obtained to determine possible fluctuations in the amount of histamine-containing substrate in the blood of each individual, since it has been shown that most of the histamine present in the blood is contained in the leukocytes.[3, 4]

The blood samples from each person were divided into 25 ml. aliquots and antigen was added as follows:

1. No antigen added (control)
2. 0.25 ml. ragweed antigen, 10,000 P.N.U. per milliliter
3. 0.25 ml. timothy antigen, 10,000 P.N.U. per milliliter
4. 0.25 ml. house dust antigen, 10,000 P.N.U. per milliliter
5. 0.25 ml. HASH* antigen, 10,000 P.N.U. per milliliter
6. 0.25 ml. oak antigen, 10,000 P.N.U. per milliliter
7. 0.25 ml. wheat antigen, 10,000 P.N.U. per milliliter
8. 0.25 ml. egg antigen, 10,000 P.N.U. per milliliter
9. 0.25 ml. cow's milk antigen, 10,000 P.N.U. per milliliter
10. 0.25 ml. chocolate antigen, 10,000 P.N.U. per milliliter
11. 0.25 ml. orange antigen, 10,000 P.N.U. per milliliter
12. 0.25 ml. tomato antigen, 10,000 P.N.U. per milliliter

After addition of antigen, all aliquots were incubated in a water bath at 37° C. for twenty minutes; then they were centrifuged and the supernatant plasma was removed by pipetting. After trichloroacetic acid precipitation, the plasma filtrates were assayed for histamine by the technique of Lowry and associates.[3] By this method, the average normal value for plasma histamine is 4.3 gamma per liter. All amounts of histamine in this article refer to the base.

During two of each patient's visits to the laboratory, immediately following the drawing of blood, intracutaneous tests were performed on the outer surface of the arm. One one-hundredth milliliter of each of the eleven antigens, in 100 P.N.U. per milliliter strength, was injected intracutaneously, using a B-D adapter No. 181P fitted to a Cooke asbestos syringe. Two skin test controls (one of 0.01 ml. of physiologic saline, the other of 0.01 ml. of 1-10,000 histamine diphosphate) were used.

At twenty minutes, the skin test response was recorded on Ektachrome 120 indoor film, and was evaluated and recorded by a negative to four plus grading method. All wheals no larger than the saline control were considered negative, those larger than the control but less than 1 cm. in diameter were one plus, those 1 cm. in diameter were two plus, those greater than 1 cm. were three plus, and all those with pseudopods were four plus.

Brief summaries of the individual clinical histories are presented in the appendix at the end of the article.

RESULTS

For the purpose of comparing histamine release with skin test results, three categories have been established arbitrarily: (1) normal controls, (2) patients

*A mixture of equal parts of extracts of four common molds—hormodendron, alternaria, spondylocladium, and helminthosporium, each in 10,000 P.N.U. per milliliter strength.

with "large" skin reactions, that is, at least one skin test result greater than two plus, and (3) patients with "small" skin reactions, that is, with no skin reaction larger than two plus. Histamine values and skin test results obtained in four normal controls and Patients 1, 2, 3, and 4 in the appendix appear in Table I.

Values determined for patients with large skin reactions (Patients 5, 7, 10, 16, and 17) appear in Table II. Results in those patients with small skin reactions (Patients 6, 8, 9, 11, 12, 13, 14, 15, and 18) appear in Table III.

TABLE I. HISTAMINE LIBERATION AND SKIN TEST REACTIONS IN NORMAL CONTROLS

ANTIGEN	PATIENT 1		PATIENT 2		PATIENT 3		PATIENT 4	
	H*	S†	H	S	H	S	H	S
Control	3	0	4	0	12	0	4	0
Ragweed	10	0	8	0	12	0	6	0
Timothy	5	0	8	0	45/7*	0	6	0
House dust	7	0	7	0	6	0	5	0
HASH	7	0	27/20‡	0	10	0	4	0
Oak	7	++	6	0	6	0	9	0
Wheat	4	0	6	0	6	0	7	0
Egg	6	0	4	0	6	0	8	0
Cow's milk	5	0	10	0	7	0	9	0
Chocolate	3	0	6	0	5	0	6	0
Orange	14	0	13	0	8	0	3	0
Tomato	6	+	44	0	22	0	7	0
Histamine 1-10,000		++		++		+++		++

*H = Histamine in gammas per liter.
†S = Skin test response.
‡Repeat determinations.

In general, the smallest histamine release values are found in Table I, the nonallergic controls, whose skin tests, with the exception of two small positive reactions of no known significance, were entirely negative. Among the control subjects, the greatest deviations of histamine release values from control plasma histamine values are found in Patient 2, (HASH and tomato) who denied allergic manifestations but had a history of mild, nonseasonal attacks of vasomotor rhinitis.

TABLE II. HISTAMINE LIBERATION AND SKIN TEST REACTIONS IN INDIVIDUALS WITH LARGE SKIN REACTIONS

ANTIGEN	PATIENT 5		PATIENT 7		PATIENT 10		PATIENT 16		PATIENT 17	
	H*	S†	H	S	H	S	H	S	H	S
Control	9		4		8		5		10	
Ragweed	166	+++	48	++++	62	++++	77	++++	49	+
Timothy	102	+++	55	++++	92	++++	44	++++	27	+
House dust	86	0	23	++	32	++	25	++	86	+++
HASH	48	++	22	++	121	+++	111	+	21	±
Oak	52	0	6	+++	32	++	69	++	18	++
Wheat	38	0	8	0	58	+++	16	0	20	0
Egg	26	0	6	0	61	+++	13	0	23	0
Cow's milk	33	0	5	+	13	++	23	0	17	0
Chocolate	16	0	9	0	5	0	13	0	18	0
Orange	35	+	1	0	5	0	24	+	23	±
Tomato	10	0	4	0	9	0	49	0	67	±
Histamine 1-10,000		++		+++		++++		+++		++++

*H = Histamine in gammas per liter.
†S = Skin test response.

TABLE III. HISTAMINE LIBERATION AND SKIN TEST REACTIONS IN INDIVIDUALS WITH SMALL SKIN REACTIONS

ANTIGEN	PATIENT 6		PATIENT 8		PATIENT 9		PATIENT 11		PATIENT 12		PATIENT 13		PATIENT 14		PATIENT 15		PATIENT 18	
	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H	S	H	S
Control	1		5		7		3		10		5		10		2		5	
Ragweed	26	+	3	+	19	+	2	0	39	++	22	++		0	29	++	22	0
Timothy	33	0	45	+	8	0	18	±	10	++	14	++	8	±	5	±	5	0
House dust	23	0	17	0	7	0	6	0	26	+	10	0	10	0	2	0	6	0
HASH	9	0	7	0	11	0	9	0	10	0	9	±	5	0	2	0	24	±
Oak	11	0	13	0	10	0	9	0	14	0	5	++	6	0	3	0	5	0
Wheat	222*	0	28	0	8	0	44	0	53	++	6	±	4	0	3	±	4	0
Egg	20	0	13	0	20	0	36	0	23	±	7	±	5	0	2	0	5	0
Cow's milk	2	0		0	14	0	47	0	64	0	5	+	9	0	2	0	8	0
Chocolate	4	0	9	0	14	0	3	0	4	0	7	0	5	0	3	0	4	0
Orange	3	0	31	0	12	0	6	0	7	++	16	±	7	0	7	0	5	0
Tomato	135*	0	26	0	14	0	9	0	53	++	10	±	8	0	8	0	30	0
Histamine 1-10,000		++		++		++		+++		+++		+++		+++		+++		+++

H = Histamine in gammas per liter.

S = Skin test response.

*These high values are thought to result from technical error. They have been disregarded in the statistical results presented and in the discussion.

In the "large" skin reaction group shown in Table II are also found the largest histamine release values. Four of these exceed 100 gammas per liter, and several others approach this amount.

Although no attempt to select ragweed-sensitive cases was made, all the patients with large skin reactions were ragweed sensitive by skin test and reacted to timothy and house dust also. Except in Patient 10 (wheat and egg), all the large skin reactions were to inhalants.

In the group with "small" skin reactions, there is likewise a predominance of inhalant reactions, with positive skin tests and significant histamine release to ragweed, timothy, and house dust antigens in a majority of the patients. However, some patients (9, 11, and 12) show larger histamine release values with food antigens than with inhalant antigens.

DISCUSSION

It is apparent that antigens other than ragweed will liberate large amounts of histamine from the blood cells of a clinically sensitive person. In general, in the patients tested, the food antigens did not liberate as much histamine as the inhalants, just as the corresponding skin tests to foods did not result in reactions as large as those seen with inhalant antigens. Nevertheless, in some instances the food antigens liberated amounts of histamine many times the control plasma histamine value.

Since it has long been appreciated that different organs of an individual vary in the degree of reaction to a given antigen, differences in the capacity of blood cells and skin to release histamine are to be expected. Further, the possibility that histamine is not the pharmacologic agent involved in the production of skin test wheals, as suggested by the work of Bowman and Walzer,[5] must be considered. In this study, however, the large histamine release values were found in those patients with large skin test reactions.

TABLE IV. AVERAGE AMOUNT OF HISTAMINE RELEASED COMPARED WITH SKIN TEST REACTIONS

DEGREE OF SKIN POSITIVITY:	++++	+++	++	+	±	NEGATIVE
Histamine Liberation*:						
"Large" skin reactors	11.5	11.2	4.6	7.7†/3.5	3.7	2.9
"Small" skin reactors			3.5	4.0†/3.4	2.0	2.4

*Figures represent histamine produced by the antigens employed for the corresponding skin tests, expressed as an average of the number of times the control histamine values.

†This average is weighted by one unusually large value in a relatively small group. Averaging the group without this figure gives the value shown immediately to the right.

Excluding the control group, if one compares the degree of positivity of the skin test reaction with the degree of histamine release, histamine release appears to diminish as the degree of skin reaction decreases. This relationship is illustrated in Table IV, where an average release number has been calculated for each skin test reaction group. The release numbers are the values of histamine liberated in terms of the control plasma histamine.

Although this fairly consistent correlation between skin test reaction and histamine release is apparent, significant amounts of histamine may be released

when skin tests are negative. Examples of clinically important antigens which released large amounts of histamine into the plasma, although the skin tests were negative, are shown in Table V.

It is difficult to evaluate the exact significance of this finding, since in several instances large amounts of histamine were released by antigens of no known clinical significance. Sufficient data are not available in this study, however, to compare the incidence of "false positive" histamine release with "false positive" skin reaction.

TABLE V. CLINICALLY IMPORTANT ANTIGENS GIVING NEGATIVE SKIN TESTS AND POSITIVE HISTAMINE RELEASE

	PATIENT 5	PATIENT 6	PATIENT 11	PATIENT 11	PATIENT 12
Antigen used	Dust	Timothy	Wheat	Milk	10
Histamine released	86	33	44	47	Milk
Control plasma histamine	9	1	3	3	64

Histamine values are in gammas per liter.

As with skin tests, "false negative" histamine release tests are apparent. Patient 14, who gave a history of asthma following exposure to dust and mold, not only had negative skin tests, but the histamine release values did not exceed the control value. A striking example of how both skin test and histamine release value may be negative with a strongly positive clinical history is found in Patient 16, who, since early childhood, has had anaphylaxis-like reactions immediately after eating egg. The patient made her most recent test of egg sensitivity one week after blood was obtained for these determinations. At that time, two minutes after she swallowed a bit of egg yolk, which in her case produces a less violent reaction than egg white, she developed hives about the mouth, a sensation of "swelling" in her throat, and slight difficulty in breathing. While it is commonly known that food antigens producing allergic symptoms may give negative skin tests,[5] the finding is particularly striking in this patient who shows large skin test reactions to other antigens.

CONCLUSIONS

Although the small number of cases studied makes it impossible to arrive at any firm generalizations, these studies suggest the following conclusions:

1. Both food and inhalant antigen of various origins, when incubated with the blood of a sensitive person, will cause the release of histamine into the plasma.

2. Those antigens producing the largest skin reactions in a person also cause the greatest release of histamine.

3. In some instances, clinically significant antigens, particularly food antigens, liberate large amounts of histamine when the skin tests are negative; in others, both skin tests and blood histamine release are negative when there is a good clinical history of sensitivity to an antigen.

APPENDIX

Histories of Patients Studied

Patient 1 is a 23-year-old nurse, whose only allergic manifestation has been an occasional appearance of a few small hives of a few hours' duration. Her father has "chronic bronchitis."

Patient 2, a 33-year-old housewife, has a history of mild, nonseasonal attacks of vasomotor rhinitis, the cause of which has never been determined. Skin tests done in the past for common foods and inhalants have been completely negative. There is no known family history of allergic manifestations.

Patient 3 is a 46-year-old housewife with no history of allergic manifestations, and no family history of allergic disease.

Patient 4, a 37-year-old physician, has no family history of allergic disease. The only known possibly allergic manifestations have been nausea, vomiting, and diarrhea following ingestion of clams on several occasions.

Patient 5 is a 24-year-old male student, who, at the age of 15 years, developed severe hay fever during the ragweed season. At times, cough and wheezing would accompany the hay fever. In January, 1952, treatment with ragweed antigen was started and has been continued. Since then he has experienced only mild rhinitis during the ragweed season. These symptoms have been well controlled with tripelennamine hydrochloride. Exposure to house dust may cause an occasional attack of sneezing and rhinorrhea during the winter. No foods are known to cause exacerbation of symptoms. A sister, brother, and nephew have hay fever.

Patient 6 is a 41-year-old housewife, who, during December, 1947, developed cough, wheezing, and shortness of breath, which continued intermittently until she was first seen in September, 1948. At that time she was having moderately severe asthma each day. The only positive skin test was a two plus reaction to 1,000 P.N.U. house dust, even though at times she exhibited a marked dermatographia. Her subsequent course has been one of moderately severe asthma, controlled fairly well by repeated courses of hormone therapy (both ACTH and cortisone). The exacerbations have occurred at any season and are sometimes initiated by respiratory infections. Many of them, however, bear no identifiable relationship to infection. Hay fever, at times accompanied by increased asthma, has been present in early June.

Patient 7, a 26-year-old student, has a history of ragweed hay fever since the age of 6 years. He denied any symptoms resembling hay fever at any other time of the year, but states that his nose is "wet" the year round. When having hay fever symptoms, he notes that ingestion of egg, chocolate, or watermelon will produce a temporary exacerbation and he avoids these foods during the ragweed season. At age 12, he had one "shot" for hyposensitization. His symptoms are well controlled with tripelennamine hydrochloride. His mother and sister have allergic rhinitis.

Patient 8 is a 36-year-old housewife with a history of hay fever during the ragweed season since childhood. Nasal polyps were removed in 1949. Skin tests in 1950 showed a four plus reaction to 100 P.N.U. ragweed extract, and a smaller (but still plus four) reaction to 100 P.N.U. house dust extract. Hyposensitization with ragweed and house dust extracts was started. In June, 1950, immediately following 400 P.N.U. of ragweed extract and 400 P.N.U. of house dust extract, generalized urticaria developed. This subsided within six hours with symptomatic treatment. Two weeks later, following an intervening treatment without reaction, she developed a similar constitutional reaction following 350 P.N.U. of each extract. Subsequent hyposensitizing treatment has been uneventful. She continues to develop symptoms during the ragweed season, and has not noted as much improvement during the past two seasons as during the first three years of treatment. Her paternal grandfather has hay fever. A maternal aunt has asthma.

Patient 9 is a 27-year-old lawyer who had wheezing with respiratory infections in childhood. In 1949 he developed perennial nasal congestion, and in August, 1951, he developed sneezing and rhinorrhea, followed in a few days by asthma. Intracutaneous skin tests at the time showed an equivocal reaction to 1-10,000 ragweed, a two plus reaction to 1-1,000 house dust, and a two plus reaction to 1-1,000 alternaria. Desensitization with house dust, alternaria, and respiratory vaccine was started. With symptomatic therapy, the asthma cleared rapidly, and the patient abandoned treatment. In the following October, when seen again, he reported "lots of hay fever and congestion in the chest" after August 15. The same pattern has

appeared in the subsequent two ragweed seasons. Skin tests to ragweed and other common weeds and to alternaria and other molds have been negative. His mother has chronic sinusitis. Two maternal uncles have asthma.

Patient 10, a 28-year-old physician, noted hay fever for the first time at the age of 18 years. His symptoms occur primarily during June and July. Since 1951, however, symptoms have extended through the summer months into October, and have become progressively more severe. On four occasions during the past six years, there have been brief episodes of dyspnea and wheezing. In infancy, the patient had a skin rash, said to be due to cow's milk. While there is no known family history of allergy, his father has "chronic bronchitis and bronchiectasis."

Patient 11, a 31-year-old crane operator, first developed "bronchitis" in 1944. In April, 1946, he was told he had asthma, and in succeeding years had attacks at any season of the year. In April, 1949, and October, 1950, nasal polyps were removed. In 1949, after eliminating white flour and cow's milk from his diet, his asthma was much improved. In 1950 it recurred, to disappear promptly when goat's milk was eliminated from the diet. Since that time he has done very well, having had no asthma during the past eighteen months. He has received dust and mold injections during this time. His father had "bronchial trouble"; his paternal grandmother had chronic urticaria and asthma.

Patient 12 is a 43-year-old housewife who has had flexural eczema, asthma, and rhinitis since infancy. Contact dermatitis has been produced by detergents, wave sets, and various ointments and lotions given her in the past. In the past twenty years, skin tests have been done many times with striking and fairly consistent results. There have been large reactions to white flour and smaller ones to chocolate and tomato. Occasionally, an equivocally positive test to egg has been noted. Flare-ups of eczema after ingestions of milk have been recorded. The inhalant producing the largest skin reaction has always been houst dust, with ragweed the second largest. At present the patient is receiving hyposensitizing injections of ragweed, house dust, oak, wool, and box elder. Her father had severe "nasal catarrh" which apparently was associated with his work as a baker. The maternal grandmother had asthma.

Patient 13, a 37-year-old housewife, developed allergic rhinitis in May, 1948. Subsequently, hay fever and, at times, asthma have appeared in April and May. During the ragweed seasons of 1949 and 1950 she had hay fever, accompanied in 1950 by moderate asthma late in the season. In May, 1950, hyposensitization with ragweed and dust extracts was started, and six months later treatment with oak extract was begun. The patient has continued to have mild rhinitis and asthma during the oak and ragweed pollen seasons and isolated brief attacks during the winter months. The patient's mother has had rhinitis and asthma.

Patient 14 is a 55-year-old minister who developed asthma at the age of 45 years. A sister had asthma in childhood. The patient has asthmatic attacks following exposure to house dust, musty smells, beef, and lettuce. In 1949, skin tests showed moderate reactions to milk and tomato and a slight reaction to house dust. In 1952, skin test reaction to oak was two plus. While under observation during the past five years, the patient has continued to have asthma at any time during the year, with the only consistent seasonal exacerbation occurring in late June and July. During the past three years, treatment with house dust, oak, and timothy pollens has seemed to be of benefit in that his attacks are not as frequent or as severe.

Patient 15 is a 38-year-old housewife who developed asthma shortly after moving from Colorado to Illinois at 12 years of age. Since then she has continued to have rather severe asthma at times, occurring at almost any season of the year. She has not been troubled by hay fever or sinusitis, and denies any seasonal exacerbation of symptoms. Because of asthma, however, she has been seen in October and November during three of the past four years. Her father had "chronic sinusitis," and a paternal aunt had asthma.

Patient 16, a 38-year-old secretary, developed eczema at 3 months of age and a few months thereafter had asthma, which was severe most of the time until after her graduation from high school. After the elimination of certain foods, flexural eczema disappeared during adolescence. In the past, egg, fresh cherries, and various other foods which have not been

identified have caused hives. From infancy, when a minute amount of egg was eaten, perioral hives appeared immediately. Larger amounts of egg have produced wheezing and generalized urticaria. During the past few years, she has developed a perioral dermatitis in April, and she has a perennial, moderately severe rhinitis and conjunctivitis. Skin tests in 1950 and 1954 showed large reactions to a number of common inhalants, but no reaction to egg.

Patient 17, a 54-year-old housewife, developed chronic urticaria in childhood. She had "bronchitis" in the winter for many years and then developed it in the summer. At the age of 22 years, while pregnant, she developed asthma for the first time. Skin tests in 1939 revealed one plus reactions to ragweed and lamb's-quarter and a four plus reaction to house dust. In 1941, the house dust reaction was three plus, and there were smaller reaction to feathers, wool, and cow dander. On two occasions in the past, she has shown a one plus reaction to egg, although reaction to egg was negative in November, 1954. She thinks that house dust causes asthma and, while she feels that she has been better on a diet eliminating positive food reactors, there is no food which consistently causes trouble. A brother and sister have "chronic sinusitis."

Patient 18 is a 46-year-old nurse who, in February, 1951, developed a severe, nonproductive cough which has persisted almost without interruption to the present. She has noted no reasonal variation in the cough, although in the late summer she has had unusual shortness of breath on exertion and "tightness of the chest." Potatoes, white bread, egg yolk, and spices seem to cause sneezing and nasal stuffiness. In childhood she had eczema and occasional attacks of wheezing. Her mother had "chronic bronchitis."

REFERENCES

1. Katz, G., and Cohen, S.: Experimental Evidence of Histamine Release in Allergy, J. A. M. A. **117**: 2, 1782, 1941.
2. Noah, J. W., and Brand, A.: Release of Histamine in the Blood of Ragweed-Sensitive Individuals, J. ALLERGY **25**: 210, 1954.
3. Lowry. O. H., Graham, H. T., Harris, B., Priebat, M. K., Marks, A. R., and Bregman, R. U.: The Chemical Measurement of Histamine in Blood Plasma and Cells, J. Pharmacol. & Exper. Therap. **112**: 116, 1954.
4. Graham, H. T., Wheelwright, F., Parish, H. H., Jr., Marks, A. R., and Lowry, O. H.: Distribution of Histamine Among Blood Elements, Federation Proc. **2**: 350, 1952.
5. Bowman, K. L., and Walzer, M.: The Response of Specific and Histamine Wheal Sites in Atopic Skins to Restimulation With Specific and Non-Specific Excitants, J. Immunol. **29**: 81, 1933.
6. Duke, W. W.: Asthma, Hayfever, Urticaria, and Allied Manifestations of Allergy, St. Louis, 1926, The C. V. Mosby Company, p. 207.

IODIDE HYPERSENSITIVITY IN THE ETIOLOGY OF PERIARTERITIS NODOSA

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INTRODUCTION

IN 1866 Kussmaul and Maier[1] first described the pathologic lesions of periarteritis nodosa, but the clinical recognition of the disease did not materialize until after the turn of the century. Gruber,[2] in 1925, was the first to suggest that hypersensitivity reactions to foreign substances might play an etiological role in this disease. The occurrence of periarteritis in patients given foreign sera[3, 4, 13, 22] and the demonstration of similar lesions developing in animals as a result of a hypersensitive immune response to foreign sera[33-38] support this concept. A similar vasculitis has been ascribed to hypersensitivity reactions from simple chemical substances,[3-19, 21-32] including iodides.[5] Table I lists these compounds.

Serious reactions have resulted from the administration of iodine, iodides, and iodine-containing compounds.[20, 39-76] These are listed in Table II. The clinical picture of some of the severe reactions was consistent with an underlying vasculitis, but description of pathologic material was lacking or was

TABLE I. POSSIBLE ETIOLOGICAL AGENTS IN CASES OF PERIARTERITIS NODOSA

1.	Serum[3, 4, 13, 22]
2.	Sulfonamides[4, 6-12, 21]
3.	Penicillin[25-28]
4.	Iodides[5, 14]
5.	Arsenicals[19]
6.	Mercury[23]
7.	Aspirin[24]
8.	Dilantin[29, 30]
9.	Phenylbutazone[31]
10.	D.D.T.[32]
11.	Thiourea, thiouracil, propylthiouracil[15-18]
12.	Phenobarbital[6]

inadequate for substantiation.[20, 40-43, 45, 68, 70, 76] Two patients with thyrotoxicosis developed periarteritis nodosa during combined thiourea and iodide treatment. In both patients, the periarteritis was ascribed to thiourea hypersensitivity, but a hypersensitive response to iodides cannot be excluded.[15, 17] In only two instances has iodide hypersensitivity been clearly implicated in the etiology of periarteritis nodosa.[5, 12]

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TABLE II. SENSITIVITY REACTIONS TO IODIDES[20, 33-70]

1. Asthma
2. Angioneurotic edema, urticaria
3. Pulmonary edema
4. Peripheral edema
5. Transitory pulmonary infiltrations
6. Thrombopenic and nonthrombopenic purpura
7. Fever
8. Eosinophilia
9. Arthritis
10. Hematuria
11. Albuminuria
12. Localized necrotizing vascular lesions
13. Various cutaneous lesions
14. Lymphadenopathy

The following cases are reported, as they may represent two further instances in which periarteritis nodosa developed as a result of iodide hypersensitivity.

CASE REPORTS

CASE 1.—The patient, R. R. (MGH #787816), was a 60-year-old woman. In August of 1932, when the patient was 40 years of age, asthma first appeared; it cleared in October. Asthma has recurred each year from early August to October. Symptoms usually responded promptly to conservative therapy which, on at least two occasions, included potassium iodide. In August, 1952, she was referred to the Massachusetts General Hospital because her asthma did not respond to the usual medications. On admission, she had severe asthma. Blood pressure was 162/92; pulse, 100; and the heart was enlarged 1 cm. beyond the midclavicular line in the fifth left intercostal space. White blood count was 10,000 per cubic millimeter, with 1 per cent eosinophils. A roentgenogram of the chest showed slight cardiac enlargement and pulmonary emphysema. Epinephrine, aminophylline, and sedatives relieved her completely in two days. She was discharged on potassium iodide, ephedrine, aminophylline, and intramuscular penicillin for five days. Ten days later, nausea followed the ingestion of potassium iodide. In spite of this, she continued taking 10 drops three times a day. Within three days, she developed abdominal bloating, a constricting sensation in the lower anterior chest, dyspnea on exertion, and ankle edema. Re-examination in the outpatient department demonstrated bilateral pleural effusions and cardiac enlargement due to a pericardial effusion. Signs and symptoms continued in spite of digitalis and Mercuhydrin. She was readmitted, therefore, on Oct. 3, 1952. Blood pressure was 170/100; temperature, 101 to 104° F.; pulse, 100. In addition, a large liver and a pulsus paradoxus of 15 mm. Hg was noted. Her white blood count was 9,000, with 29 per cent eosinophils. Electrocardiographic changes were late inversion of the T waves in Leads I, II, aV_L, V_{4-6}, notched P waves, and an upright T wave in aV_R. On October 8, an objective arthritis developed in the right hand and wrist, and four days later a generalized petechial rash appeared. Platelet count was normal. On October 16, a pericardial tap was performed because of signs of increasing cardiac tamponade; 400 c.c. of serosanguineous fluid was removed, the sediment of which contained 40 per cent eosinophils. A muscle biopsy performed on October 18 showed a perivascular inflammatory reaction in one area and monocytic and eosinophilic infiltration in several other areas. The pathologist interpreted these findings as being consistent with periarteritis nodosa. These lesions are illustrated in Figs. 1 and 2. An eight-day course of intravenous ACTH, 25 mg. a day, resulted in a marked increase in appetite and a feeling of well-being; disappearance of eosinophilia, petechial rash, fever, and arthritis; and decrease in edema and heart size. Improvement persisted for two weeks after cessation of ACTH. Fever recurred, as well as a slight eosinophilia (6 to 10 per cent). In addition, a right radial nerve neuritis and left-sided Jacksonian seizures appeared. Dilantin and cortisone (300 mg. daily) therapy was started

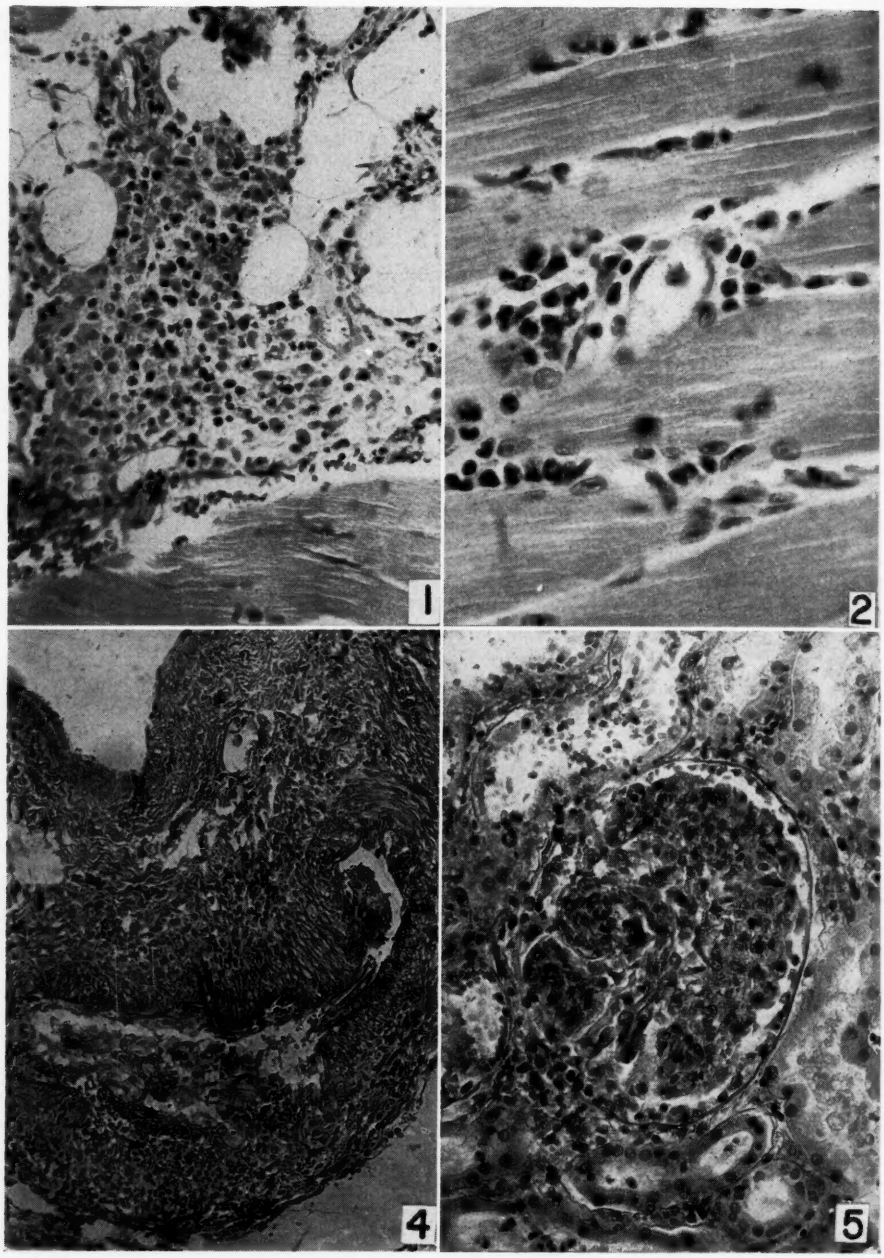


Fig. 1.—Patient R. R. An inflammatory infiltrate in skeletal muscle composed of monocytes, lymphocytes, and eosinophils. (Magnification, ×200; reduced ⅛.)

Fig. 2.—Patient R. R. A small blood vessel, in skeletal muscle, surrounded by lymphocytes and plasma cells. (Magnification, ×400; reduced ⅛.)

Fig. 4.—Patient N. D. A marked inflammatory reaction around and in the walls of a small arteriole in the mesentery of the colon. (Magnification, ×200; reduced ⅛.)

Fig. 5.—Patient N. D. A glomerulus, showing partial destruction of Bowman's capsule, proliferation of glomerular tufts, and thrombosis of some of the capillaries. (Magnification, ×200; reduced ⅛.)

at once. Within three days, signs and symptoms disappeared, but were followed by the development of a left hemiparesis, which gradually improved. Dilantin and cortisone were continued for three months. For the next two years, the patient was carefully followed and she has been well except for asthma during the months of August through October. A repeat muscle biopsy on Jan. 28, 1954, revealed no diagnostic abnormality.

Although the first muscle biopsy was not completely diagnostic of periarteritis, it was consistent with this diagnosis and similar to lesions ascribed to this disease.[5, 77, 78] The diagnosis was established on the basis of these findings and the clinical course.

CASE 2.—The patient was N. D. (MGH #816443), a 37-year-old woman, who first noted asthma in May, 1953, when she was 36 years of age. Recurrent attacks persisted for the next month, and became progressively more severe, in spite of therapy. She came to the Massachusetts General Hospital on July 1, 1953, with intractable asthma. Treatment with sedatives, epinephrine, and aminophylline failed, and on the third hospital day an eleven-day course of cortisone was started. She obtained complete relief. Laboratory studies, including a roentgenogram of the chest, were normal. She remained well except for rare, mild, easily controlled attacks of asthma until June, 1954, when increasingly severe and persistent asthma

Fig. 3.—A photograph of the hands of patient N. D. On the dorsum of the left hand was a purpuric eruption which had an erythematous base and superimposed hemorrhagic vesicles. The last two fingers of the right hand could not be completely extended as a result of a radial nerve palsy.

reappeared. She returned to the hospital on July 4, 1954. On entry, she had severe asthma which responded promptly to epinephrine and aminophylline, but one week later she returned because of another attack which also responded rapidly. Routine laboratory studies and a chest roentgenogram were normal. Potassium iodide, 10 drops three times daily, was instituted on July 16, 1954. Within twenty-four hours, recurrent giant urticaria appeared and was followed by the appearance, during the next two weeks, of fever, malaise, arthritis, and muscle pains. All previous therapy was stopped on July 30 and she was given a two-week course of cortisone in decreasing daily doses after an initial dose of 300 mg. Two weeks later, while still taking 50 mg. of cortisone a day, these symptoms returned. In

addition, during the next two weeks she developed, in succession, nausea, bloody diarrhea, purpura, conjunctivitis, muscle pains, stabbing pains in the forearm, and weakness progressing to partial paralysis of the hands.

When readmitted on Aug. 30, 1954, it was determined that on two previous occasions (June, 1953, and June, 1954) she had developed periorbital edema, joint pains, and fever after the ingestion of an "asthma pill" found to be enteric-coated potassium iodide.

Two years prior to this admission, she had undergone a hysterectomy. Review of the pathologic material revealed no vascular disease.

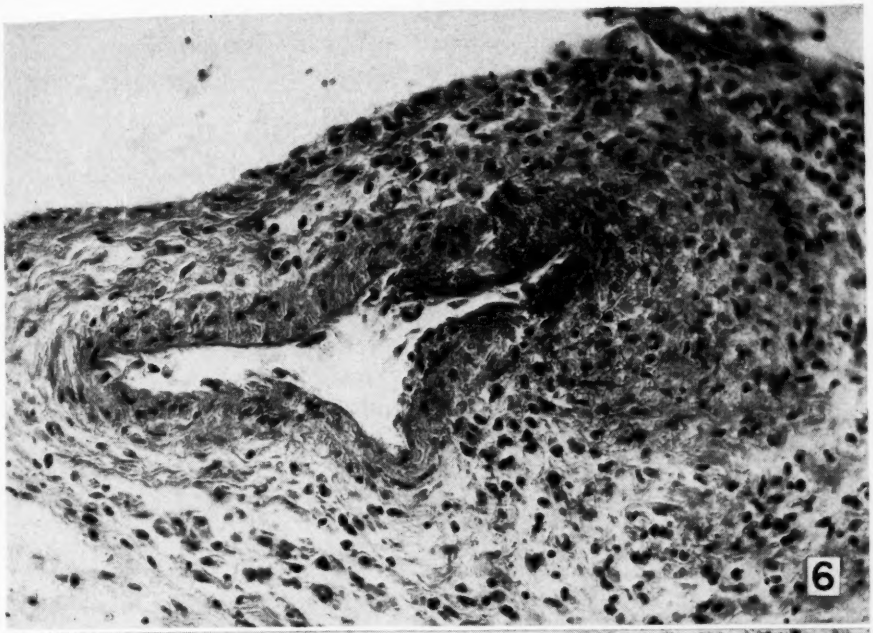
The important physical findings were temperature 100.4° F.; pulse, 110; blood pressure, 108/70; petechiae on the conjunctival and buccal mucosae; moist râles at the right lung base; right upper quadrant tenderness; a bilateral radial nerve neuritis, including motor and sensory loss; an absent right ankle jerk; ankle edema; and a generalized dermatitis, maximal on the distal extremities, consisting of urticaria, petechiae, ecchymoses, and vesicles. A photograph of the skin lesions is shown in Fig. 3.

Laboratory studies revealed hemoglobin, 11.3 Gm. per cent; white blood count, 14,000 per cubic millimeter, with 14 per cent eosinophils; minimal microscopic hematuria; a positive stool guaiac; a normal platelet count; and an abnormal serum electrophoretic pattern, with low albumin and high gamma globulin. Sigmoidoscopy revealed extensive submucosal nonulcerating purpura. Electrocardiogram was abnormal, with S-T segment depression and low T waves in Leads II, III, aV_F, and V_6. A roentgenogram of the chest showed a localized infiltration in the right lower lobe. Using the technique of Prausnitz and Küstner, the patient's sera were tested with Benadryl, aspirin, and potassium iodide. One-tenth cubic centimeter of the patient's serum was injected intracutaneously and the sites were challenged twenty-four hours later. Benadryl gave a positive control, so the results were of no value. Aspirin gave a questionable reaction in one of two subjects. A 1:20 dilution of potassium iodide, applied as a scratch test, gave an immediate type reaction (flare and wheal within twenty minutes) in both recipients. Precipitating antibodies were not demonstrated in the patient's serum, using as antigen either potassium iodide or serum from a patient ingesting this compound.

Initially, the patient seemed to improve on 6.5 Gm. of aspirin a day, but by the sixth hospital day the hemoglobin had fallen to 8.5 Gm. per cent, and eosinophilia had risen to 54 per cent. She was transfused with two units of whole blood, and promptly developed pulmonary edema. At the same time, thymol iodide was applied inadvertently to a small decubitus ulcer. In spite of all measures (including gitalin, compound F, and positive-pressure oxygen), pulmonary edema continued, she became hypotensive, and developed azotemia, increasing cardiac enlargement, and progressive widespread electrocardiographic changes consistent with severe myocardial ischemia and infarction. She died on the eleventh hospital day.

There were widespread changes at postmortem examination. The dermatitis was as previously described. The heart weighed 325 grams. Nearly the entire left ventricle, the septum, and the inferior portion of the right ventricle were infarcted. There was an overlying fibrinous pericarditis. The coronary vessels were soft and uninvolved with atherosclerosis. A pericardial effusion of 125 c.c. and bilateral pleural effusions, 400 c.c. each, were present. The lower lobe of each lung contained a hemorrhagic consolidation. The upper lobes were markedly edematous. Multiple small cerebral infarcts were seen in the area of distribution of the right posterior cerebral artery. There was a widespread hemorrhagic ulcerating process involving the mucosa of the colon, terminal ileum, and gall bladder.

The microscopic findings were striking. There was extensive necrosis of the walls of the heart, with areas of inflammatory infiltrate composed of neutrophils, lymphocytes, plasma cells, and eosinophils, the latter predominating. The inflammatory reaction was seen in the myocardium, endocardium, and epicardium. Mural thrombi were present over the endocardial surface of the left ventricle. There were several areas of focal necrosis



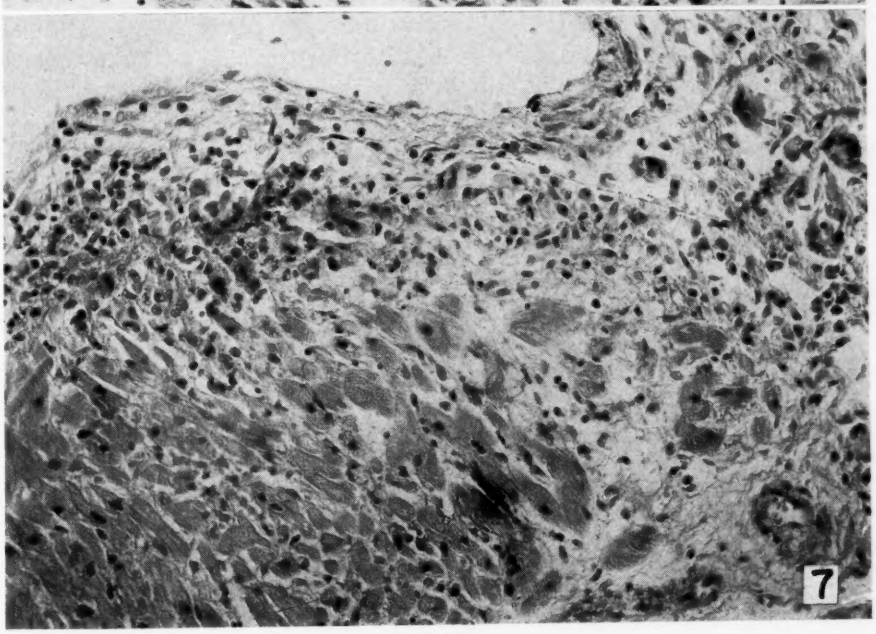


Fig. 6.—Patient N. D. A typical lesion in a coronary arteriole, showing fibrinoid necrosis of the vessel wall and perivascular inflammation. (Magnification, ×200; reduced ⅛.)

Fig. 7.—Patient N. D. A section of myocardium containing multinucleated giant cells on the right and an area of fibrinoid necrosis with surrounding inflammatory cells on the left. (This lesion had many of the characteristics of an Aschoff body.) (Magnification, ×200; reduced ⅛.)

with surrounding inflammatory reaction and multinucleated giant cells in the myocardium. These resembled Aschoff's nodules. Widespread involvement of the small arterioles (250 μ or less) was present in the pancreas, heart, peripheral nerves, colon, skeletal muscle, gall bladder, kidneys, and stomach, and less so in the lungs. The lesions were of several types:

1. Occlusion of arterioles with fibrin thrombi of various ages.
2. Focal collections of inflammatory cells perivascularly without inflammation of the vessel wall. (These collections of cells were of two types—in certain areas they were predominantly lymphocytes, while in others they were neutrophils, plasma cells, and eosinophils.)
3. Fibrinoid necrosis of vessel walls with perivascular inflammation, and destructive inflammation of all three layers of the vessel wall.

Similar lesions were seen in a few venules. The bone marrow showed eosinophilic hyperplasia and was otherwise normal. Both kidneys contained focal granulomatous lesions composed of monocytes, plasma cells, neutrophils, lymphocytes, and eosinophils. Focal necrosis and fibrin thrombi in capillaries with proliferation of endothelial cells were seen in some glomeruli. The lungs contained edema and a hemorrhagic organizing pneumonia, in which there were areas of necrosis and eosinophilic infiltration. Photomicrographs of some of these lesions are shown in Figs. 4, 5, 6, and 7.

DISCUSSION

The serum sickness type of hypersensitivity can be induced by the injection of foreign sera.[3, 22, 24, 35] A similar state can occur following the use of some simple chemical substances. Pathologically, damage to small blood vessels has been described. This ranges from reversible, visually undetectable changes resulting in local edema formation to irreversible, necrotizing vasculitis with resultant organ dysfunction.[22, 35-38, 77]

The occurrence of periarteritis nodosa in some patients is thought to represent such a severe, irreversible vasculitis.[4, 79-81] The exact pathogenesis of this vasculitis remains undetermined. A clinical and pathologic differentiation of these cases of so-called hypersensitivity angiitis from other cases of periarteritis nodosa has been proposed,[79-82] but not generally accepted.[83-85] The present cases may represent instances of a vasculitis of this type.

Since asthma may be a manifestation of periarteritis nodosa,[73, 86] it is possible that both patients had periarteritis prior to iodide therapy, the asthma being an initial symptom of the disease. In each patient, however, the course of the disease altered dramatically shortly after the institution of iodides. In neither patient was asthma present during the course of the vasculitis, suggesting that the asthma and the vasculitis resulted from hypersensitivity reactions to different agents. The presence of asthma in the first patient, at a time when all signs of the vasculitis had disappeared, is consistent with the preceding interpretation, as is the clinical course. In the second patient, the past history of iodide sensitivity, the presence of skin-sensitizing antibodies to iodide, and the seasonal nature of the asthma support the theory of separate etiologies. A recrudescence of the vasculitis occurred in each after the cessation of a short course of steroid therapy, even though no iodides had been administered in the meantime. This is compatible with iodide hypersensitivity

being an etiological factor, since patients ingesting potassium iodide have elevated, nonthyroxine, blood, protein-bound iodine, which may remain three to four weeks after cessation of iodide administration.[87, 88]

Accepting iodide hypersensitivity as an etiological factor, it is reasonable to think that periarteritis may develop in other patients with bronchial asthma as a result of therapy with iodides or possibly other agents and, further, that the etiological agent may be overlooked if asthma is accepted as a symptom of the vasculitis. This is of some practical importance, as recognition and elimination of the sensitizing agent are therapeutically desirable. Iodides are used extensively in asthma therapy; yet the occurrence of vasculitis following their use is rare. This would indicate that either (1) such an occurrence is truly a rare phenomenon; (2) it occurs more frequently than suspected, but goes unrecognized; or (3) the severe, clinically serious form is rare, but less severe manifestations occur frequently and go unnoticed. The recognition of such early signs of sensitivity in the second patient might have been lifesaving. Abandonment of iodide therapy cannot be recommended on the basis of this evidence, but withdrawal of such treatment in patients developing any minor manifestations of sensitivity is clearly indicated.

If vasculitis of this nature can develop from iodide hypersensitivity, the mechanism of its production is of interest. Administration of simple chemical compounds can result in the development of hypersensitivity reactions.[89] Basically, the sequence of this development is thought to be: (1) the union of agent with plasma protein, forming an antigen; (2) stimulation of specific antibody production by this antigen; (3) union of antigen with antibody, resulting in vascular damage.[89]

All substances, including iodides, proposed as etiological agents in cases of periarteritis (Table I) have been shown to form a complex with plasma proteins, when such studies have been carried out.[90] There has been intensive study of iodoproteins, including their immunologic properties. It is important, in discussing these, to distinguish iodine (I^0) from iodide (I^-), since their interactions with proteins differ. These are illustrated in Fig. 8 and are discussed. Iodide is the reduced form of iodine. Except in the thyroid gland, this substance exists in vivo in the reduced state (I^-), regardless of the route of administration,[91-95] with the one exception that subcutaneously administered iodine may exist, as such, for some time.[91] These facts have an important bearing on the interpretation of the results of the immunologic studies and their relation to human iodide sensitivity.

Most of the immunologic studies have employed, as antigens, protein treated in vitro with iodine (I^0) solutions. Under these conditions, the combination of iodine (I^0) with protein involves either substitution on the phenolic group of tyrosine, or on the imidazole group of histidine, or addition on the thio-ether linkage of methionine.[96] Iodination may cause the disappearance of the (OH) group of diiodotyrosine.[97] The only one of these reactions which seems to have an important bearing on the problem of altered antigenicity is the substitution of iodine on tyrosine. Proteins iodinated in this way alter

their serologic specificity to a greater or lesser degree, depending on the percentage of iodine added.[79, 89, 98-101] With "complete" substitution of the tyrosine groups, nearly complete loss of the original specificity occurs and a common specificity with other iodinated proteins appears.[98] From the nature of the changes in the protein molecule, iodide inhibition of the antigen-antibody reactions of these iodoproteins would not be expected. The precipitation of antibodies from the sera of rabbits sensitized by iodoproteins can be blocked by diiodotyrosine but not by potassium iodide.[99] Similarly, anaphylaxis in sensitized guinea pigs can be blocked by diiodotyrosine but not by potassium iodide.[100]

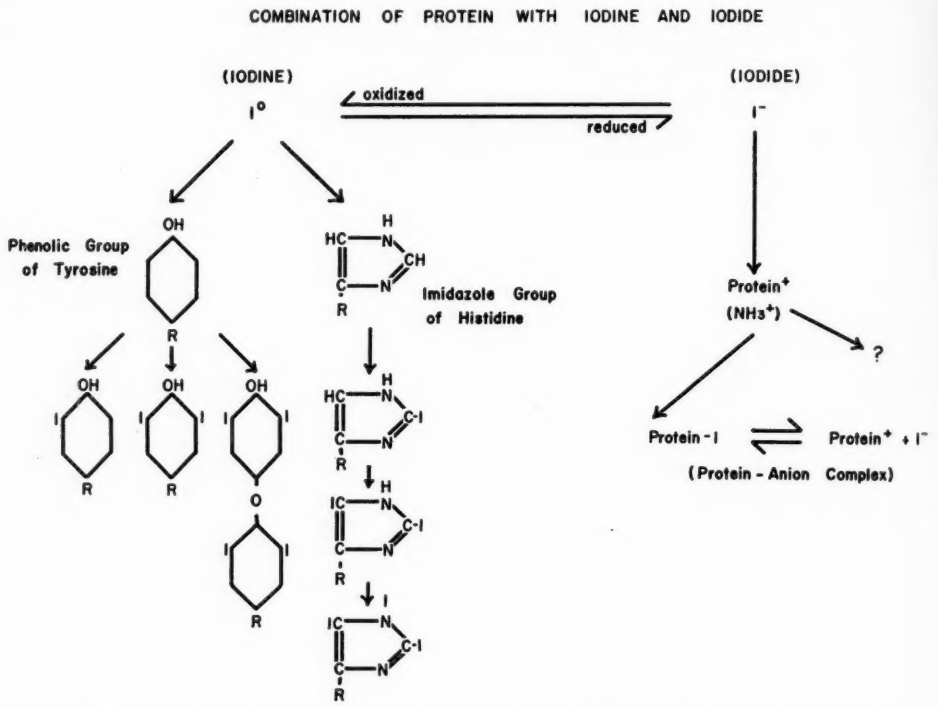


Fig. 8.—The interaction of protein with iodine (I^0) and iodide (I^-). Iodine combines with tyrosine to form mono- and di-iodotyrosine, and thyroxine. Its addition to histidine takes place in three successive steps, as illustrated. Iodide is ionically bound to protein, and may combine in other, undefined ways.

The interaction of iodide (I^-) with proteins has not been thoroughly studied. Small anions, such as chloride and thiocyanate are bound in vitro by certain unidentified groups of human serum albumin.[102, 103] No data are available for iodide, but similar binding might be expected, from theoretical considerations. Iodide does form a complex with serum in vitro and 8 to 31 per cent of this bound iodide is protein precipitable.[104] The in vivo binding of iodide to serum albumin does occur in patients ingesting potassium iodide,[87, 88] but the nature of this binding has not been investigated. This albumin-iodide complex is also, in part at least, protein precipitable. It is

conceivable that the iodides have been oxidized to iodine, and the latter substituted onto tyrosine radicals. This is not likely, however, since the in vitro iodination of serum produces a calorigenic substance (tyrosine-substituted protein),[105] and the in vivo production of a similar substance might be expected if iodide were converted to iodine. Such a substance has not been found. On the basis of the available data, it is reasonable to propose that the antigen responsible for the development of iodide sensitivity in man is a protein-iodide complex.

The demonstration of the skin-sensitizing antibodies to iodides, in the serum of the one patient, would be consistent with this proposal. The relationship of these antibodies to the development of the vasculitis cannot be defined. However, there is some experimental evidence equating such antibodies with the anaphylactoid state. Guinea pigs, sensitized to simple chemical substances, produce circulating, nonprecipitable antibodies which, when appropriately challenged, can induce either the immediate type of skin sensitization or passive anaphylaxis.[106]

Assuming that sensitivity to iodides can produce a spectrum of anaphylactoid manifestations ranging from angioedema to severe vasculitis, and that the production of these is contigent upon a protein-iodide complex becoming antigenic, several problems remain unsolved. Does every person ingesting iodide form the same protein-iodide complex? If so, what factors determine when and in whom it becomes antigenic? What defines the severity and location of the ensuing sensitivity reaction? Further fruitful study of these problems might be undertaken using the readily available radioactive iodine I^{131}.

A discussion of sensitivity to iodine compounds would not be complete without mention of two other aspects. Sialorrhea, bronchorrhea, tearing, and salivary gland enlargement are described as common reactions to iodide administration.[107] From our present knowledge, it would seem that these symptoms may represent a heightened physiologic or pharmacologic effect of the drug, and not true sensitization. Many reactions occur with the use of iodine containing contrast substances in radiographic procedures.[66] The iodized oil, such as that used in bronchography is probably unreactive until swallowed and absorbed through the gastrointestinal tract or absorbed directly through the lung. In either case, the mechanism of sensitization is probably a combination of liberated iodide with plasma protein. Organic compounds, as used in pyelography, for example, probably become antigenic by combining with plasma proteins directly, rather than releasing iodide.

SUMMARY

Two patients with periarteritis nodosa are described. In both, iodide hypersensitivity was implicated as an etiological factor. Skin-sensitizing antibodies to potassium iodide were found in the serum of one patient. The significance of this finding is discussed. Possible mechanisms of iodide sensitivity are presented.

REFERENCES

1. Kussmaul, A., and Maier, R.: Über Eine Bisher Nicht Beschreibene Eigenthumliche Arterienkrankung (Periarteriitis Nodosa), die mit Morbus Brightii und Rapid Fortschreitender Allgemeiner Muskellahmung Einhergeht, Deutsches Arch. klin. Med. **1**: 484, 1866.
2. Gruber, G. B.: Zür Frage der Periarteriitis Nodosa, mit Besonderer Berucksichtigung der Gallenblasen und Nieren-Beterligung, Arch. path. Anat. **258**: 441, 1925.
3. Rich, A. R.: The Role of Hypersensitivity in Periarteritis Nodosa, as Indicated by Seven Cases Developing During Serum Sickness and Sulfonamide Therapy, Bull. Johns Hopkins Hosp. **71**: 123, 1942.
4. Rich, A. R., and Gregory, J. E.: The Experimental Demonstration That Periarteritis Nodosa Is a Manifestation of Hypersensitivity, Bull. Johns Hopkins Hosp. **72**: 65, 1943.
5. Rich, A. R.: Hypersensitivity to Iodine as a Cause of Periarteritis Nodosa, Bull. Johns Hopkins Hosp. **77**: 43, 1945.
6. Lichtenstein, L., and Fox, L. J.: Necrotizing Arterial Lesions, Resembling Those of Periarteritis Nodosa, and Focal Visceral Necrosis Following Administration of Sulfathiazole, Am. J. Path. **22**: 665, 1946.
7. More, R. H., McMillan, G. C., and Duff, G. L.: The Pathology of Sulfonamide Allergy in Man, Am. J. Path. **22**: 703, 1946.
8. French, A. J.: Hypersensitivity in the Pathogenesis of the Histopathologic Changes Associated With Sulfonamide Chemotherapy, Am. J. Path. **22**: 679, 1946.
9. Black-Schaffer, B.: Pathology of Anaphylaxis Due to Sulfonamide Drugs, Arch. Path. **39**: 301, 1945.
10. Goodman, M. J.: Periarteritis Nodosa With Recovery, Report of an Unusual Case Apparently Due to Sensitivity to Sulfadiazine, Ann. Int. Med. **28**: 181, 1948.
11. Gelfand, M. L., and Arnonoff, S.: Periarteritis Nodosa, Possible Relation to Increased Usage of Sulfonamides, Ann. Int. Med. **30**: 919, 1949.
12. Thompson, R. T., and Zeek, P.: Acute Necrotizing Angiitis Due to Hypersensitivity Following Sulfonamide Therapy, Ohio M. J. **41**: 824, 1945.
13. Eason, J., and Carpenter, G.: Treatment of Acute Rheumatic Polyarthritis With Concentrated Anti-Scarlatinal Serum, Quart. J. Med. **6**: 93, 1937.
14. Muller, C.: Periarteritis Nodosa—Asthma Bronchiale—Iododerma Tuberosum, Acta med. scandinav. **136**: 378, 1949-50.
15. Gibson, P. C., and Quinlan, J. T.: Periarteritis Nodosa in Thiourea Therapy, Lancet **2**: 108, 1945.
16. Dalgleish, P. G.: Polyarteritis Nodosa After Thiouracil, Lancet **2**: 319, 1952.
17. Barnum, D. R., de Takatas, G., and Dolkart, R. E.: Periarteritis Nodosa Following Thiouracil Therapy of Hyperthyroidism: Resultant Hypertension Benefited by Sympathectomy, Angiology **2**: 256, 1951.
18. McCormick, R. V.: Periarteritis Occurring During Prophyl-thiouracil Therapy, J. A. M. A. **144**: 1453, 1953.
19. Miller, H. G., and Nelson, M. G.: Polyarteritis Nodosa Developing During Anti-Syphilitic Treatment, Lancet **2**: 200, 1945.
20. Barker, W. B., and Wood, W. B., Jr.: Severe Fatal Iodism During Treatment of Hyperthyroidism, J. A. M. A. **114**: 1029, 1940.
21. Rich, A. R.: Additional Evidence of the Role of Hypersensitivity in the Etiology of Periarteritis Nodosa: Another Case Associated With Sulfonamide Reaction, Bull. Johns Hopkins Hosp. **71**: 375, 1942.
22. Clark, E., and Kaplan, B. I.: Endocardial, Arterial, and Other Mesenchymal Alterations Associated With Serum Disease in Man, Arch. Path. **24**: 458, 1937.
23. Friedenthal, M., and Cagan, R. N.: Periarteritis Nodosa Associated With the Use of Proprietary Mercurial Preparation, Am. Pract. & Digest Treat. **3**: 218, 1952.
24. Rich, A. R.: Hypersensitivity in Disease, Harvey Lect. **42**: 106, 1946-47.
25. Waugh, D.: Myocarditis, Arteritis, and Focal Hepatic, Splenic, and Renal Granulomas Apparently Due to Penicillin Sensitivity, Am. J. Path. **28**: 437, 1952.
26. Berne, R. M.: An Unusual Sensitivity Reaction to Penicillin, New England J. Med. **242**: 814, 1950.
27. McCombs, R. P.: Drug Allergies: Case Reports of Unusual Sequelae in Three Patients, Bull. New England M. Center **13**: 39, 1951.
28. Surdakowski, A. Z.: Periarteritis Nodosa Due to Penicillin, New York J. Med. **54**: 388, 1954.
29. Rothermuch, N. O.: Polyarteritis Nodosa: A Review Together With Report of a Case Due to Hydantoin Sensitization Treated With Cortisone, Ohio M. J. **47**: 1013, 1951.

30. VanWyk, J. J., and Hoffman, C. R.: Periarteritis Nodosa: A Case of Fatal Exfoliative Dermatitis Resulting From "Dilantin Sodium" Sensitization, Arch. Int. Med. **81**: 605, 1948.
31. O'Brien, D. J., and Storey, G.: Death From Hypersensitivity, Brit. M. J. **1**: 792, 1954.
32. Hill, W. R., and Damiani, C. R.: Death Following Exposure to D.D.T.: Report of a Case, New England J. Med. **235**: 897, 1946.
33. Germuth, F. G., Jr.: A Comparative Histologic and Immunologic Study in Rabbits of Induced Hypersensitivity of the Serum Sickness Type, J. Exper. Med. **97**: 257, 1953.
34. Rich, A. R.: The Occurrence of Focal Tuberculoid Lesions in Experimental Serum Sickness, Bull. Johns Hopkins Hosp. **91**: 109, 1952.
35. Fox, R. A., and Jones, L. R.: Vascular Pathology in Rabbits Following Administration of a Foreign Protein, Proc. Soc. Exper. Biol. & Med. **55**: 294, 1944.
36. Hawn, C. V., and Janeway, C. A.: Histological and Serological Sequences in Experimental Hypersensitivity, J. Exper. Med. **85**: 571, 1947.
37. Hopps, H. C., and Wessler, R. W.: The Experimental Production of Generalized Arteritis and Periarteritis (Periarteritis Nodosa), J. Lab. & Clin. Med. **31**: 939, 1946.
38. Ehrich, W. E., Seifter, J., and Forman, C.: Experimental Serum Disease, J. Exper. Med. **89**: 23, 1949.
39. Snodgrass, W. R.: Observations on the Therapeutic Use of Iodides, Quart. J. Med. **4**: 247, 1935.
40. Newman, E. A., and Ross, P. H.: Thyrotoxicosis Complicated by Severe Iodism; Preparation for Surgery With Propylthiouracil, J. Clin. Endocrinol. **7**: 212, 1947.
41. Hollander, L., and Fetterman, G. H.: Fatal Iododerma: The Eleventh Case Reported in the Literature, Arch. Dermat. & Syph. **34**: 228, 1936.
42. Eller, J. J., and Fox, E. C.: Fatal Iododerma, Arch. Dermat. & Syph. **24**: 745, 1931.
43. Mi, C. H., and Yang, C. T.: Iodide Fever, Chinese M. J. **52**: 887, 1937.
44. Davis, W. C., and Saunders, T. S.: Purpura Due to Iodides, Arch. Dermat. & Syph. **53**: 644, 1946.
45. Hed, A. R.: Jodallergi, Nord. med. **46**: 1371, 1951.
46. Ehrich, W. E., and Seifter, J.: Thrombotic Thrombopenic Purpura Caused by Iodine, Arch. Path. **47**: 446, 1949.
47. Cleveland, D. E. H.: Iododerma From Asthma Remedy, Canad. M. A. J. **30**: 181, 1934.
48. Seymour, W. B., Jr.: Poisoning From Cutaneous Application of Iodine, Arch. Int. Med. **59**: 952, 1937.
49. Denning, H.: Thrombopenische Purpura Nach Jodeinnahme, München. med. Wchnschr. **80**: 562, 1933.
50. Katzenstein, L.: Fever Due to Iodides, Am. J. Syph., Gonor. & Ven. Dis. **22**: 346, 1938.
51. Usher, B.: Iododerma Associated With Loeffler's Syndrome, Canad. M. A. J. **64**: 67, 1951.
52. Hyde, J. N.: A Contribution to the Study of the Bullous Eruption Induced by the Ingestion of the Iodide of Potassium, Arch. Dermat. **5**: 333, 1897.
53. Guptill, P.: Acute Iodism During Treatment of Toxic Goiter, Surgery **11**: 962, 1942.
54. Jacobs, J. L., and Colmes, A.: Cutaneous Hypersensitivity to Iodine, J. Lab. & Clin. Med. **26**: 302, 1940.
55. Snell, N. W., and Savin, L. H.: Iodism in Which Edema of Glottis Necessitated Tracheotomy, Lancet **1**: 759, 1927.
56. Rutherford, W. J.: Excessive Reaction Following the Use of Tincture of Iodine, Brit. M. J. **1**: 846, 1915.
57. Clifford, S. H.: Iodine Hypersensitivity, Boston M. & S. J. **195**: 931, 1926.
58. Alstead, S.: Iodism, Glasgow M. J. **123**: 196, 1935.
59. Wartzki, I. M.: Acute Iodide Eruption Following the Ingestion of a Small Amount of Potassium Iodide, M. J. Australia **2**: 739, 1938.
60. Spencer, H. A.: Anaphylaxis Resulting From the Use of an Iodide, J. Trop. Med. **33**: 339, 1930.
61. Felsher, Z.: The Nature of Halogen Sensitivity in Dermatitis Herpetiformis and Pemphigus, J. Invest. Dermat. **8**: 55, 1947.
62. Pelner, L.: Iodine Allergy, J. Lab. & Clin. Med. **27**: 1150, 1942.
63. Hand, E. A.: Temporary Unilateral Loss of Vision Associated With Iododerma, Arch. Dermat. & Syph. **54**: 29, 1946.
64. Sheldon, J. M., and Lovell, R. G.: Asthma Due to Halogens, Am. Pract. **4**: 43, 1949.
65. Sulzberger, M. B., and Wilten, V. H.: Allergic Dermatoses Due to Drugs, Postgrad. Med. **11**: 549, 1952.
66. Irwin, F. M.: Iodism, Ann. Otol., Rhin. & Laryng. **61**: 408, 1952.
67. Mackenzie, S.: Fatal Purpura From Iodide of Potassium, Illust. Med. News **1**: 169, 1888-89.

68. Wilson, J. H.: Purpura Haemorrhagica From Iodide of Potassium, Brit. M. J. 1: 470, 1889.
69. Robinson, T.: Rapid Production of Purpura After Small Doses of Iodide of Potassium, Lancet 1: 471, 1893.
70. Cape, R. D. T.: Iododerma of the Face and Marked Eosinophilia, Brit. M. J. 1: 255, 1954.
71. Walker, N.: Iododerma, or Dermatitis Tuberosa, Due to the Ingestion of Iodide of Potassium, Lancet: p. 571, 1892.
72. Lindsay, S. S.: Acute Edema of the Face Due to Potassium Iodide, Lancet 2: 217, 1919.
73. Rackemann, F., and Greene, F.: Periarteritis Nodosa and Asthma, Tr. Am. A. Physicians 54: 112, 1939.
74. Joffe, J. J.: Erythema Iodicum Bullosum Haemorrhagicum With Fatal Termination, Arch. Dermat. u. Syph. 163: 517, 1931.
75. Bechet, P. E.: Role of Iodized Table Salt in Iododerma, J. Invest. Dermat. 8: 409, 1947.
76. Alcan, D. N.: Sensitivity to Iodides and Bromides in Dermatoses Other Than Dermatitis Herpetiformis, J. Invest. Dermat. 7: 287, 1947.
77. Churg, J., and Strauss, L.: Allergic Granulomatosis, Allergic Angiitis and Periarteritis Nodosa, Am. J. Path. 27: 277, 1951.
78. Ophiils, W.: Periarteritis Acuta Nodosa, Arch. Int. Med. 32: 870, 1923.
79. Zeek, P. M.: Periarteritis Nodosa: A Critical Review, Am. J. Clin. Path. 22: 777, 1952.
80. Zeek, P. M., Smith, C. C., and Weeter, J. C.: Studies on Periarteritis Nodosa; Differentiation Between Vascular Lesions of Periarteritis Nodosa and of Hypersensitivity, Am. J. Path. 24: 889, 1948.
81. Blankenhorn, M. A., and Knowles, H. C., Jr.: Periarteritis Nodosa: Recognition and Clinical Symptoms, Ann. Int. Med. 41: 887, 1954.
82. Zeek, P. M.: Periarteritis Nodosa and Other Forms of Necrotizing Angiitis, New England J. Med. 248: 764, 1953.
83. Rich, A. R.: Personal Communication.
84. Castleman, B. J.: Personal Communication.
85. Nuzum, J. W.: Polyarteritis Nodosa, Arch. Int. Med. 94: 942, 1954.
86. Wilson, K. S., and Alexander, H. L.: The Relationship of Periarteritis Nodosa to Bronchial Asthma and Other Forms of Human Hypersensitivity, J. Lab. & Clin. Med. 30: 195, 1945.
87. Danowski, T. S., Johnston, S., and Greenman, J. H.: Alterations in Serum Iodine Fractions Induced by the Administration of Inorganic Iodide in Massive Dosage, J. Clin. Endocrinol. 10: 519, 1950.
88. Danowski, T. S., Mateer, F., Wergard, F. A., Peters, J. H., and Greenman, J. H.: Serum Iodine Fractions in Subjects Receiving Potassium Iodide in Small Dosage, J. Clin. Endocrinol. 10: 532, 1950.
89. Landsteiner, K.: The Specificity of Serological Reactions, Cambridge, 1945, Harvard University Press.
90. Goldstein, A.: The Interaction of Drugs and Plasma Protein, Pharmacol. Rev. 1: 102, 1949.
91. Salter, W. T.: The Endocrine Function of Iodine, Cambridge, 1943, Harvard University Press.
92. Riggs, D. S.: Quantitative Aspects of Iodine Metabolism in Man, Pharmacol. Rev. 4: 284, 1952.
93. Cole, V., Dunn, R., and Curtis, G.: The Intrapulmonic Absorption of Iodine, J. Pharmacol. & Exper. Therap. 53: 327, 1935.
94. Cohn, B. N. E.: Absorption of Compound Solutions of Iodine From the G. I. Tract, Arch. Int. Med. 49: 950, 1932.
95. Sollman, T.: The Fate of Iodin, Iodids, and Iodates in the Body, J. Pharmacol. & Exper. Therap. 9: 269, 1917.
96. Roche, J., and Michel, R.: Natural and Artificial Iodoproteins, Advances Protein Chem. 4: 253, 1951.
97. Cohn, E. J., Salter, W. T., and Ferry, R. J.: Society Proceedings, J. Biol. Chem. 123: 24, 1938.
98. Wormall, A.: Immunological Specificity of Proteins, J. Exper. Med. 51: 295, 1930.
99. Jacobs, J.: Serological Studies on Iodinated Sera I: Precipitins and Precipatinogens, J. Immunol. 23: 361, 1932.
100. Jacobs, J.: Serological Studies on Iodinated Sera. II: Anaphylaxis, J. Immunol. 23: 375, 1932.
101. Klecykowski, A.: Quantitative Investigation of Changes in Antigenic Properties in the Course of Iodination of Horse-Serum Globulin, Brit. J. Exper. Path. 21: 98, 1940.

102. Scatchard, G., Scheinburg, I. H., and Armstrong, S., Jr.: Physical Chemistry of Protein Solutions. IV: The Combination of Human Serum Albumin With Chloride Ion, J. Am. Chem. Soc. **72:** 535, 1950.
103. Scatchard, G., Scheinburg, I. H., and Armstrong, S. Jr.: Physical Chemistry of Protein Solutions V: The Combination of Human Serum Albumin With Thiocyanate Ions, J. Am. Chem. Soc. **72:** 540, 1950.
104. Wyngaarden, J. B., Stanbury, J. B., and Dutoit, C. H.: On the Mechanism of Iodide Accumulation by the Thyroid Gland, J. Clin. Endocrinol. **11:** 1259, 1951.
105. Lerman, J., and Salter, W. T.: Iodinated Protein in Human Athyreosis, J. Clin. Invest. **18:** 493, 1939.
106. Chase, M. W.: Studies on the Sensitization of Animals With Simple Chemical Compounds X: Antibodies Inducing Immediate-Type Skin Reactions, J. Exper. Med. **86:** 489, 1947.
107. Means, J. H.: The Thyroid and Its Diseases, Philadelphia, 1948, J. B. Lippincott Company.

DERMATITIS VENENATA FROM TREE POLLEN OILS

A Clinical Report

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DERMATITIS venenata from exposure of a sensitized person to plant oils has been recognized for many years.[1, 2] Poison ivy (*Rhus toxicodendron*) dermatitis has been so common and troublesome that it is well known to all. Ragweed pollen, probably the most common cause of hay fever in the United States, also has contributed to some extent to the dermatitis venenata problem. Dermatitis of the exposed areas of the skin may be caused by hypersensitivity to the ether-soluble fraction of the pollen and the plant leaves and stem. This clinical syndrome has been adequately discussed with complete references by Fromer and Burrage.[3]

The possibility that the ether-soluble fraction of plant oil from other plants also could cause similar disease is assumed.[4] Trees (particularly the conifers, whose sap is used for preparing pitch and turpentine) have contributed to the contact dermatitis problem. The observation of hypersensitivity to turpentine in carpenters, painters, and woodcutters is not rare, but the existence of contact sensitivity to tree *pollen* oil first was brought to our attention in 1951 through a visit to our clinic by an unusual patient. She consulted the University Hospital in May, stating that she ran a gasoline service station in northern Michigan and that her problem involved a dermatitis which appeared on the face, hands, and forearms every April and May when the trees began to blossom. The skin lesions were distributed on the exposed areas and were clearing at the time of her visit. They were erythematous, scaling, and excoriated, and the physician who saw her felt that she had contact dermatitis. She was patch tested to the available testing materials, which consisted of juniper, turpentine, oil of cade, and pine oil. None of these gave a positive reaction, and she was discharged with symptomatic treatment. The following April she sent a letter to the clinic, stating that her dermatitis had returned "according to schedule," and asked if anything new had been discovered which would be of help. As a result of this, the need for a complete set of tree oil patch testing materials became apparent.* She was requested to come in for testing but did not return.

The similarly of the history given a few weeks later by a 68-year-old farmer (Case 2, Mr. A. M.) from upper Michigan was striking. He stated that for twenty years, since being a farmer, he had noted a dermatitis on the exposed

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*These patch testing materials can be obtained from Graham Laboratories, 6146 Willow Lane, Dallas, Texas.

areas of his skin only during the fall and in late April and May. His skin promptly cleared after May and he had no further difficulty until fall, during September and October. Then no trouble occurred until spring, when the maple, elm, and birch trees began to blossom. When he came south 300 miles to visit his son on March 29, 1953, his symptoms came about ten days earlier than

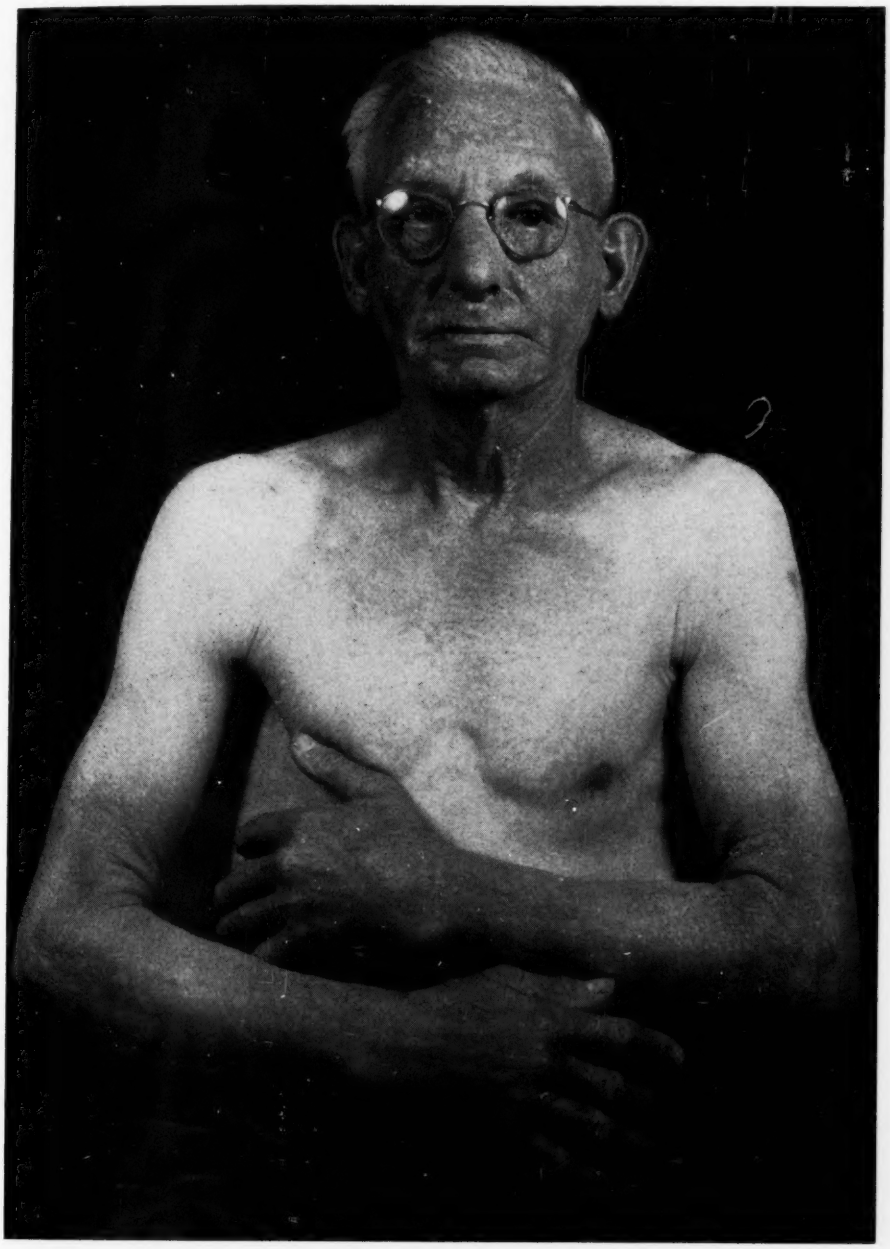

Fig. 1.—Case 2, A. M. Dermatitis confined to exposed areas.

expected. This difference corresponded with the earlier pollination of trees in the Detroit area. The patient was seen on April 20, stating that four days after arrival in the Detroit area his skin flared up and his eyes watered and itched. There was no sneezing or itching of the nose. The first trouble with his skin had occurred in 1933, following the commercial handling of bananas, and was characterized by an eczematoid dermatitis of the face, palms, and forearms, which cleared a few months after he left the fruit business. He subsequently also found it necessary to avoid orchard spray, since this irritated his skin. Except for urticaria (cause unknown) in the fall of 1952, he had no collateral allergy. There was no family history of allergy.

TABLE I. PARTIAL LIST OF PATCH TESTS APPLIED TO PATIENT 2, MR. A. M.

MATERIAL	DILUTION	RESULT
1. Copper sulfate	1 per cent aqueous	−
2. Sodium arsenate	10 per cent aqueous	−
3. Potassium dichromate	0.5 per cent aqueous	−
4. Bichloride of mercury	1:1000 aqueous	−
5. Nickel chloride	5 per cent aqueous	−
6. Tincture of Pyrethrum	20 per cent tincture	−
7. Butesin picrate	1 per cent in petrolatum	++++
8. Paraphenylenediamine	2 per cent in petrolatum	+++
9. DDT	50 per cent in olive oil	−
10. Para-red, deep #1	powder	++++
11. Para-red, deep #2	powder	++++
12. Para-red, light	powder	++++
13. Eosin dye	powder	++++
14. Ragweed oil	1:1000 in olive oil	+/−
15. Poison ivy oil	1:5000 in olive oil	+/−
16. Maple pollen oil	1:100 in olive oil	+
17. Box elder pollen oil	1:100 in olive oil	++
18. Poplar pollen oil	1:100 in olive oil	−
19. Oak pollen oil	1:100 in olive oil	−
20. Cedarwood oil	1:100 in olive oil	−
21. Birch pollen oil	1:1000 in olive oil	−
22. White ash pollen oil	1:100 in olive oil	−
23. Quack grass oil	1:100 in olive oil	+
24. Black-eyed Susan oil	1:100 in olive oil	+
25. Olive oil (control)	1:1	−

Scratch and intradermal tests with the defatted (protein) portion of the tree pollens listed above were all negative.

Examination in 1953 showed that he had a scaling, erythematous dermatitis on his face, neck, forearms, and hands; the palms were clear (Fig. 1). In consultation with a dermatologist, a tentative diagnosis of contact sensitivity to bananas, tree spray, and plant oils was made, and he was treated with starch and soda baths, calamine lotion in 50 per cent peanut oil, adrenalin in oil 1:500, and repeat action Chlor-Trimeton. His lesions cleared in about ten days and it was felt safe to patch test him with the newly obtained ether-soluble tree pollen oils. This was done by the usual technique[5, 6] (Table I) and in about twelve hours positive reactions appeared at the testing sites and increased in severity until, by thirty-six hours, several had become vesicular (Fig. 2). Control tests using the same materials on normal persons were negative. In addition, the patient was patch tested with sixty common plant oils and showed +1 reactions to *Agropyron repens* (quack grass) *Anthemis catula,* and *Rudbeckia hirta* (black-eyed Susan) and equivocal reactions to ragweed and poison ivy oils. A known

sensitivity to red paint was confirmed by patch testing with farm machinery red paint, and he was warned to avoid handling red-covered magazines and red cardboard containers which might contain para-red dyes.

Since oral desensitization to plant oils has been reported to be successful in some cases of ragweed pollen oil sensitivity,[7] it was decided to prepare an extract containing equal parts of ragweed, maple, and box elder pollen oil diluted with equal parts of olive oil and made into 1:50, 1:500, 1:5,000, 1:50,000 and 1:500,000

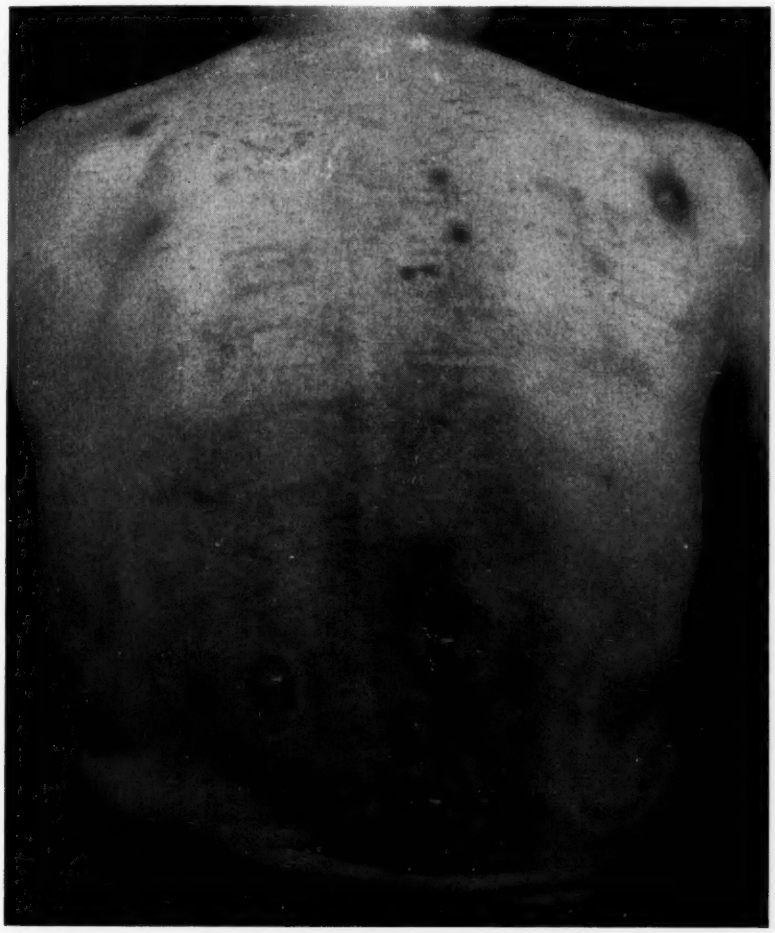

Fig. 2.—Case 2, A. M. Patch test reactions. *Upper left:* tree pollen oils. *Upper right:* Butesin picrate and paraphenylenediamine. *Lower right:* para red dyes.

dilutions. This was done and the patient was discharged, after being instructed in the technique of taking the extract. It was seen that he could tolerate the 1:500,000 dilution orally without reaction. He was to start with one drop of the 1:500,000 material on a cracker with meals once daily, building up gradually in the usual manner to fifteen drops and working up through the various dilutions to a maintenance dose of the highest dilution tolerated. He reported by

letter in June, 1953, that he had tolerated his medication initially, but that six drops of the 1:5,000 material had caused a breaking out of his skin in the same area as before. He discontinued the plant oil and his skin cleared. His extract was diluted tenfold and he resumed the oil after reassurance. He reported in November, 1953, by letter that his skin again had flared, and he would not continue the treatment in spite of our entreaties. His local physician prescribed treatment which cleared his lesions initially, but subsequently he has had intermittent and persistent flare-ups of dermatitis. He was seen again in March, 1954, with a generalized vesicular, weeping, erythematous dermatitis which was felt to be due to the application of several proprietary remedies. Skin biopsy showed hyperplasia of the epidermis with some increase in keratin and increase of melanin in the basal layer. He was discharged in April, 1954, considerably improved.

The third case was that of a 65-year-old retired policeman who previously had a diagnosis, made in 1951, of plant oil contact dermatitis from ragweed oil and *Leptilon canadense* (mare's tail). His history extended over twenty years. Dermatitis of the face, arms, hands, and legs began each year in the last two weeks of August and cleared by frost. The skin lesions were typical of plant oil dermatitis, with a vesicular, pruritic, erythematous rash located over the exposed areas. Relief was obtained by his remaining in the basement. Patch tests to the sap of stems, leaves, and pollen oil of ragweed and mare's tail, both of which were observed near his home, were positive, showing vesiculation. Oral desensitization to these plant oils was begun in May, 1951, and was progressing satisfactorily. He could tolerate mare's tail in a concentration of 1:1,000 and

TABLE II. PARTIAL LIST OF PATCH TESTS APPLIED TO PATIENT 3, MR. E. P.

MATERIAL	DILUTION	RESULT
1. Maple pollen oil	1:100 in olive oil	++++
2. Poplar pollen oil	1:100 in olive oil	++++
3. Box elder pollen oil	1:100 in olive oil	++++
4. Ash pollen oil	1:100 in olive oil	++++
5. Elm pollen oil	1:100 in olive oil	++++
6. Oak pollen oil	1:100 in olive oil	–
7. Cedarwood oil	1:100 in olive oil	–
8. Birch pollen oil	1:100 in olive oil	–
9. Ragweed oil	1:100 in olive oil	++++
10. *Leptilon canadense* oil	1:100 in olive oil	++++
11. Olive oil (control)	1:1	–

Scratch and intradermal tests with the defatted (protein) portion of the pollens listed above were all negative.

ragweed oil in 1:100, but more concentrated extract caused dermatitis. In January, 1952, his skin was completely clear and he was taking his plant oils. He had done quite well, reporting the "best fall I've had in twenty years." However, in the spring of 1952, on May 1, he reported that he was having a flare-up of his dermatitis "out of season," which had started on April 14. Although he had been on a well-tolerated maintenance dosage of ragweed and mare's tail, this was discontinued. His symptoms did not abate. It was noted that he had severe vesicular erythematous dermatitis of the exposed areas, particularly his face and cheeks. He was treated with Phenergan, 12.5 mg. twice daily, and

Wise's shake lotion in 40 per cent peanut oil. By the first week of May he had begun to improve and finally his skin cleared. His oral desensitization was resumed. He did well through the fall and winter of 1952 and 1953, taking maintenance dosage up to 1:1,000. Between April 1 and April 14, 1953, he had a flare-up of dermatitis, starting on the legs below the knees. The face, neck, and hands were also involved. He was patch tested to tree pollen oils (Table II). Positive reactions were seen to pollen oils of elm, maple, poplar, box elder, and ash (Fig. 3). Scratch and intracutaneous tests with the protein extract of the

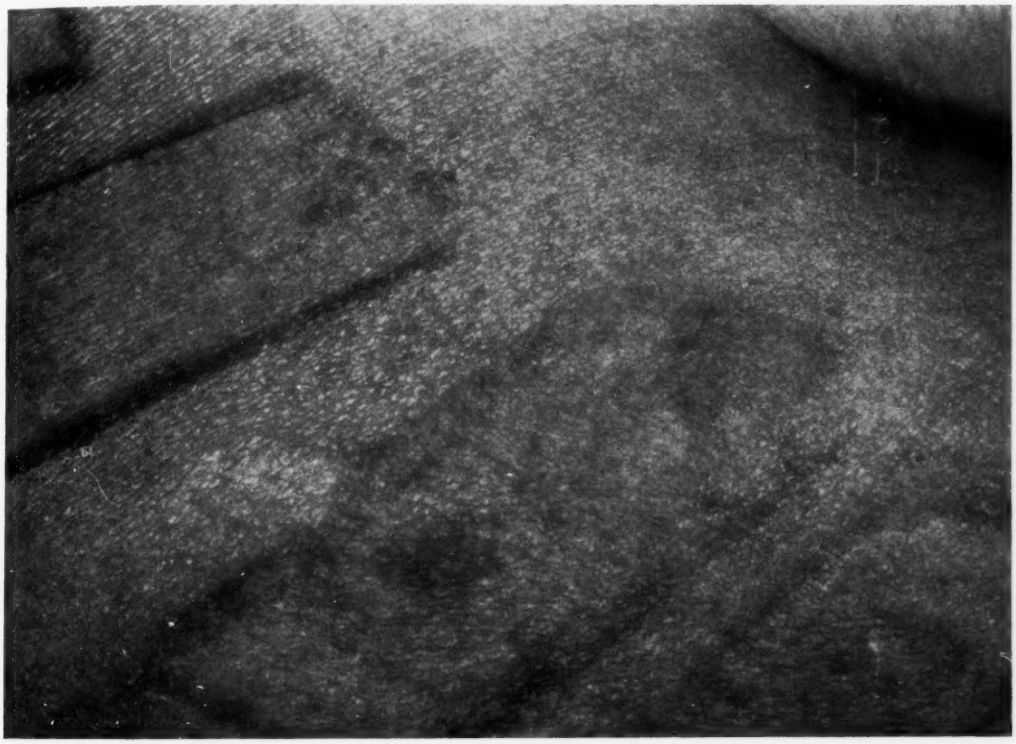

Fig. 3.—Case 3, E. P. Positive patch test reaction to tree pollen oil.

tree pollens were negative. He then was treated topically with Wise's shake lotion and cortisone, 100 mg. orally per day. Cortisone was tapered off rapidly because of a coincident hypertension. The skin cleared with this treatment by mid-May. Oral hyposensitization was begun on June 24, 1953, in a manner described in the first case, to equal parts of maple, poplar, ash, and elm. By the seventh dose he had a mild flare-up, but the program was resumed at a lower dosage level. At present the patient is on a maintenance dose of 1:500 dilution of the tree oils. If he increases dosage above seven drops of 1:50 the skin flares.

DISCUSSION

Contact dermatitis due to the ether-soluble portion of tree pollen oil is a very unusual condition. Of necessity, it would have to be a seasonal entity at first,

since the offending pollen is in the air for relatively short periods of time, a specific type being recorded in terms of days and weeks. It would be expected that the skin should clear after spring pollination was ended and remain so until the following season. The exposed areas of the skin would be involved, and the lesions would be expected to be indistinguishable from other types of dermatitis venenata. This was the case in the patients presented here. Positive patch test reactions to the tree pollen oils were observed, but there was no evidence clinically of sensitivity to tree pollen through inhalation. Patient A. M., described in this report, did complain of itching eyes with his dermatitis. Skin tests to the aqueous pollen extracts by scratch and intracutaneous methods did not confirm protein sensitivity. Careful trial of hyposensitization with the pollen oil is worth trying if symptomatic treatment is not adequate.

SUMMARY

1. Pollen oil dermatitis is a recognizable entity. Dermatitis venenata due to tree pollen oil appears to be extremely rare.

2. Two cases of dermatitis venenata are reported, and observations are made on a third case in which the symptoms occurred coincident with the tree pollination period in Michigan. These patients had typical lesions of plant dermatitis on the exposed areas of their skin. The two patients tested gave positive patch reactions with the ether-soluble fraction of the tree pollens.

3. Oral hyposensitization with pollen oil caused an exacerbation out of season in the dermatitis of both patients, causing one patient to discontinue treatment. The second person treated by oral hyposensitization to tree pollen oil now is tolerating it well.

4. Testing for possible sensitivity to plant pollen oils in dermatitis venenata might well include representatives of tree pollen oils.

REFERENCES

1. Hannah, L.: Ragweed Dermatitis—A New Treatment, J. A. M. A. **22**: 853, 1919.
2. Sutton, R. L.: Ragweed Dermatitis, J. A. M. A. **73**: 1433, 1919.
3. Fromer, J. L., and Burrage, W. S.: Ragweed Oil Dermatitis. J. ALLERGY **24**: 425, 1953.
4. Mackoff, S., and Dahl, A.: A Botanical Consideration of the Weed Oleoresin Problem Minnesota, Med. **34**: 1169, 1188, 1951.
5. Shelmire, B.: Contact Dermatitis From Weeds: Patch Testing With Their Oleoresins, J. A. M. A. **113**: 1085, 1939.
6. Sheldon, J. M., Lovell, R. G., and Mathews, K. P.: A Manual of Clinical Allergy, Philadelphia, 1953, W. B. Saunders Company, p. 190.
7. Fisher, A. A.: Some Immunologic Phenomena in Treatment of and Patch Testing for Ragweed Oil Dermatitis. J. Invest. Dermat. **19**: 271, 1952.

PENICILLIN REACTIONS IN CHILDREN

A Study of the Value of the Skin Test in Penicillin Sensitization

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With continued widespread use of penicillin, the problem of sensitization to it, with resulting symptoms, has become a distinct possibility. Since Waldbott's[1] report, in 1949, of death in an adult following an intramuscular injection of penicillin, there have been additional reports of such deaths by Mayer and associates,[2] Siegal and associates,[3] Christenson and co-workers,[4] Feinberg and his associates,[5] and others. These authors also presented cases of anaphylactic-like reactions to penicillin, with recovery. We have been able to find only one recorded death in a child following penicillin injection,[6] although we have knowledge of two such unrecorded deaths. It is interesting to note that, in the majority of instances recorded in the literature, of penicillin anaphylaxis in adults, the immediate skin reaction with penicillin has been positive. On the other hand, the skin test usually has been negative in the delayed or serum sickness type of reaction following penicillin administration.

Levin and Moss[7] state that sensitization to penicillin in children is uncommon. These authors did not observe definite local or constitutional reactions in 226 allergic children who had received such injections on more than one occasion. Only one child in their series had a transient pruritic eruption. Collins-Williams and Vincent,[8] from a study conducted in a hospital where thousands of children had received penicillin injections frequently, found only three children who were clinically sensitive to this antibiotic. Only one of these three children was skin tested with penicillin, and the result was negative. These same authors, however, in a study of children from their private practice, report that 6 per cent of allergic children gave histories of having had symptoms suggestive of penicillin sensitization as contrasted to 0 per cent of nonallergic children. They concluded, from their studies of cases in the hospital as well as from those in private practice, that the skin test had very limited value in determining clinical sensitivity to penicillin in children.

The primary purpose of our study was to evaluate the immediate reaction to the skin test with penicillin, in determining the presence of clinical sensitivity to it. To our knowledge, there is no reliable immunologic test at present to predetermine such sensitivity.

MATERIALS AND METHODS

Four hundred patients, including 397 children (between the ages of 1 year and 14 years) and three adult members of the hospital staff, were skin

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tested as outlined in Tables I and III. In all instances, the scratch test with aqueous sodium penicillin G in concentrations of 5,000 to 50,000 units per milliliter was performed first and when a negative result was obtained, the intracutaneous test followed. For the intracutaneous test 0.02 to 0.05 ml. of a freshly prepared aqueous solution of penicillin G was injected, in concentrations varying from 100 to 1,000 units per milliliter.

Weaker concentrations of penicillin were always used in those cases presenting a history of some type of clinical reactions to previous penicillin contact. The skin test with *Penicillium notatum* was performed in a similar manner, although the intracutaneous test frequently replaced the scratch test. For the intracutaneous test, 0.02 to 0.05 ml. of a 1-1000 solution was used routinely. For the scratch test with procaine, a 2 per cent solution of procaine hydrochloride was used. For the intracutaneous test, 0.02 to 0.05 ml. of an aqueous solution of procaine hydrochloride was used. We did not deem it advisable to test procaine sensitivity by its application to mucous membranes. Control tests were done with the diluent used for the preparation of all test materials. The skin testing of those children who had been hospitalized because of constitutional symptoms following penicillin administration was done when these children had recovered from these symptoms.

Passive transfer testing, in those patients who had positive skin tests with penicillin, was done with the usual technique, using well children as receptors. No concentrated study for delayed skin reactions was planned in this series of cases.

The grading of skin reactions was done in order to give the reader a visual image of the degree of skin reactivity to penicillin. Thus, the following criteria were kept in mind for the scratch test:

++++ = Large wheal, more than 1 cm. in diameter, usually with pseudopodia with or without marked areola or localized itching.
+++ = Wheal about 1 cm. in diameter, with or without pseudopodia. Moderate or marked areola usually present.
++ = Intermediate skin reaction between + and +++.
+ = Definite area of erythema as compared to control.
Negative = Same as control.

Similar criteria were applied in grading the intracutaneous tests, except that qualifications for gradings corresponding to the scratch test were more finite and less elastic.

Analysis of Table IA.—We obtained one positive test to penicillin in the entire group of 391 children routinely tested. Three hundred and fifty-three, or 90 per cent, of the children tested had previous penicillin contact by various routes of administration, including intramuscular injection in each instance. In routine taking of histories on these children, minor, delayed symptoms following administration of this antibiotic were disclosed in fourteen, or approximately 4 per cent, of the cases. Such symptoms were usually in the form of generalized skin eruptions or isolated urticaria. In no instance could we be certain, however, that these symptoms were caused by penicillin, since these

children had acute infections and the penicillin given was only one of several antibiotics or drugs given at about the same time. There were nineteen positive skin tests to *Penicillium notatum* in the 341 children tested with this mold. There were 106 children, in our series studied, who had histories of manifestations of major allergy, including bronchial asthma and allergic rhinitis. The single positive skin test to penicillin occurred in one of the children in this group. We were not able to demonstrate any positive skin tests to procaine.

TABLE IA. SKIN TESTS FOR PENICILLIN SENSITIVITY IN CHILDREN

	TOTAL NO. OF CHILDREN TESTED	PREVIOUS PENICILLIN CONTACT	NO. WITH POSITIVE SCRATCH TESTS TO PENICILLIN	NO. WITH POSITIVE INTRACUTANEOUS TESTS† TO PENICILLIN	TOTAL POSITIVE SKIN REACTORS TO PENICILLIN	POSITIVE SKIN TESTS TO PROCAINE	POSITIVE SKIN TESTS TO PENICILLIUM NOTATUM
*Hospitalized Children**							
Nonallergic group	235	215	0	0	0	0	9
Allergic group	6	4	0	0	0	0	2
Total	241	219	0	0	0	0	11
Outpatient Children							
Nonallergic group	50	48	0	0	0	0	Not done
Allergic group	100‡	86	0	1	1	0	8
Total	150	134	0	1	1	0	8
Grand total	391	353	0	1	1	0	19
Percentage of total		90 per cent			0.25 per cent		5.4 per cent

*Hospitalized for medical or surgical conditions.

†Includes presence of bronchial asthma, allergic rhinitis, and eczema (atopic).

‡Sixty cases from Pediatric Allergy Clinic, Michael Reese Hospital, and forty children from the private practice of Dr. Matheson.

TABLE IB. SKIN TESTS FOR PENICILLIN SENSITIVITY IN A SELECTED GROUP OF CHILDREN HOSPITALIZED BECAUSE OF ACUTE SYSTEMIC REACTIONS FOLLOWING PENICILLIN ADMINISTRATION

TOTAL NO. OF CHILDREN TESTED	PREVIOUS PENICILLIN CONTACT	NO. WITH POSITIVE SCRATCH TESTS TO PENICILLIN	NO. WITH POSITIVE INTRACUTANEOUS TESTS* TO PENICILLIN	TOTAL POSITIVE SKIN REACTORS TO PENICILLIN	POSITIVE SKIN TESTS TO PROCAINE	POSITIVE SKIN TESTS TO PENICILLIUM NOTATUM	POSITIVE ALLERGY HISTORY
6	6	3	2	5	0	0	4

*Intracutaneous tests done only where scratch tests were negative.
For details of cases, see Table II.

Analysis of Table II.—The table includes seven children. Six of the seven children presented were admitted to Sarah Morris Hospital as emergency cases because of severe constitutional reactions following intramuscular

TABLE II. ANALYSIS OF SYSTEMIC REACTIONS TO PENICILLIN AND SKIN REACTIONS TO PENICILLIN IN SAME

PATIENT	TYPE OF PENICILLIN AND MODE OF ADMINISTRATION	PREVIOUS PENICILLIN ADMINISTRATION AND HISTORY OF PREVIOUS REACTIONS	CLINICAL REACTIONS ON ADMISSION TO HOSPITAL	SKIN TESTS					PASSIVE TRANSFER PENICILLIN	ALLERGY HISTORY
				SCRATCH		INTRACUTANEOUS				
				PENICILLIN	PROCAINE	PENICILLIN	PROCAINE	PENICILLIUM		
1. M. A.,* 13-year-old girl	Crystalline procaine penicillin, 300,000 U., intramuscularly	Multiple; always intramuscularly. 1 week before had peculiar sensation in mouth following intramuscular injection of crystalline penicillin, also feeling of generalized itching and burning at same time	10 minutes following intragluteal injection of procaine penicillin began to experience pruritus, sweating, tightening in chest, and wheeze followed by generalized urticaria and edema of face	0	0	0	0	0	None done	Asthma, eczema, skin sensitivity to molds, house dust, etc.
2. T. G.,* 14-year-old girl	Crystalline procaine penicillin, 300,000 U., intramuscularly	Multiple intramuscular, also orally many times; no history of previous reactions obtained	About 10 days following intramuscular injection of procaine penicillin, patient developed marked laryngeal edema with stridor and generalized giant urticaria, lasted 1 week	+++ Accompanied by generalized pruritus and weakness	0	Not done	0	0	0	None known
3. S. R.,* 23-month-old boy	Crystalline procaine penicillin, 300,000 U., intramuscularly	Had one previous intramuscular injection of penicillin known to us; no history of previous penicillin reactions known	15 minutes following intramuscular injection of procaine penicillin, patient had generalized urticaria, swelling of face, laryngeal edema, and stridor, which lasted 3 days	0	0	++ (50 U.)	0	0	None done	Skin rash from orange juice, urticaria from aspirin
4. R. K.,* 15-month-old boy	Procaine penicillin with aluminum monostearate and peanut oil—recently	Penicillin injections intramuscularly (oral ?); no history of previous reactions obtained	3 days following intramuscular penicillin injection, patient developed severe generalized urticaria of entire body and face and hoarse cry	+++	0	-	0	0	++	None known

5. S. M. R.,* 9-year-old girl	Crystalline procaine penicillin, 300,000 U., intramuscularly	Two previous injections of procaine penicillin intramuscularly; also 8 to 10 oral doses of penicillin; no history of previous reactions obtained	About 10 days following intramuscular injection of procaine penicillin, child depeloped marked edema of face and extremities: also had urticaria of face and body; lasted one week	0	0	++++ (50 U.)	0	0	None done	Bronchial asthma, positive skin reactions to molds, house dust, and foods
6. H. G.,* 7-year-old boy	Crystalline procaine penicillin 300,000 U., intramuscularly	Multiple intramuscular and oral penicillin on many occasions; generalized itching following injection and undue local swelling and itching	15 minutes after intramuscular injection of procaine penicillin, patient experienced tightness in chest and wheezing, followed by generalized urticaria, and edema of the eyes	+++	0	-	0	0	+++	Bronchial asthma, pollen hay fever, and atopic dermatitis
7. G. F., 6-year-old boy (clinic case)	None	None; no history of any penicillin contact obtainable	None, past or present	?	-	+++ (500 U.)	-	+++	None done	Allergic rhinitis, bronchial asthma, positive skin reactions to molds and house dust

NOTE: Intracutaneous testing was performed only in cases where scratch testing was negative.
CONTROL: Skin tests with diluent were negative on all cases tested.
*Children admitted to hospital as urgent cases because of the severe systemic reaction.

TABLE III. ANALYSIS OF SYSTEMIC REACTIONS TO PENICILLIN IN THREE ADULTS

PATIENT	TYPE OF PENICILLIN AND MODE OF ADMINISTRATION	PREVIOUS PENICILLIN ADMINISTRATION AND HISTORY OF PREVIOUS REACTIONS	SKIN TESTS					PASSIVE TRANSFER	ALLERGY HISTORY
			SCRATCH		INTRACUTANEOUS				
			PENICILLIN	PROCAINE	PENICILLIN	PROCAINE	PENICILLIUM	PENICILLIN	
1. S. H., 26-year-old female nurse's aid	Crystalline procaine penicillin, 300,000 U., intramuscularly	Multiple injections of crystalline penicillin and procaine penicillin in peanut oil, with aluminum monostearate; history of generalized pruritus and urticaria 10 days following the last injection of procaine penicillin	+++ Accompanied by generalized pruritus	0	–	–	0	++	None known
2. R. K., 47-year-old male physician	Crystalline procaine penicillin, 300,000 U., intramuscularly	Multiple injections of procaine penicillin; also oral penicillin on several occasions; 3 days after intramuscular injection of procaine penicillin in deltoid area, patient developed pruritus, burning, and edema of hands and feet, accompanied by chills and fever to 101° F.; lasted 3 days	+++ Accompanied by generalized pruritus and edema at site of test	0	–	–	0	0	Perennial allergic rhinitis, skin sensitivity to alternaria, etc.
3. C. H., 33-year-old male physician	Procaine penicillin in sesame oil 300,000 U., intramuscularly	Multiple courses of oral and inhalant penicillin; 5 days after injection of procaine penicillin, patient developed pruritus, generalized burning and urticaria which was progressive and involved especially the extremities; lasted 12 days	+.+	0	–	0	0	+++	None known

injections of penicillin by private physicians at the patients' homes. All patients except one (Case 7) had either immediate (Cases 1, 3, and 6), accelerated (Case 4), or delayed (Cases 2 and 5) systemic reactions following such penicillin contact. It is questionable whether Case 2 can fit into the delayed or serum sickness type of reaction *at the time of our testing* or, rather, into the anaphylactic type, since this child evidenced an immediate, constitutional reaction from the scratch test with aqueous penicillin G. It rather suggests a transition stage from the last delayed to the present immediate type of reaction.

Four of the patients who had clinical as well as skin sensitivity to penicillin were distinctly allergic children and three of the four gave histories of having bronchial asthma or allergic rhinitis. It is noteworthy that in all cases, except Case 7, the skin test to *Penicillium notatum* was negative. The occurrence of a positive skin test to aqueous penicillin G in Case 7, who gave a history of no penicillin contact in any form, is difficult to explain. This child has bronchial asthma, is skin-sensitive to house dust, alternaria, Penicillium, and many foods. She is being hyposensitized in the Pediatric Allergy Clinic to house dust and alternaria.

These patients who presented systemic reactions responded readily to treatment with Adrenalin and cortisone, with or without antihistamines. In a few instances, the urticaria persisted for ten to twelve days.

The three cases in Table III represent adults on our children's hospital staff. They are not included in the statistical analysis of the child groups, but are presented for the interesting data revealed in our study of their skin reactivity to penicillin. These adults were well aware of their clinical sensitivity to penicillin because of past reactions from contact with this antibiotic.

DISCUSSION

Though reports in the literature on anaphylactic reactions following penicillin administration to children are scarce, such reactions do occur and, in our experience, are not uncommon. Such reactions may be so sudden in onset and so severe in character that the physician who witnesses them, in all probability, will reduce appreciably his indications for penicillin administration. It is well to remember that even the delayed or serum sickness type of reaction can be very severe in character, particularly in children, in whom there is a marked tendency for the occurrence of laryngeal or subglottic edema as a part of such a reaction. Accidental intravenous entrance of penicillin should be considered as a possible cause for such immediate reactions in some instances and may have occurred in Case 1 (Table II) when the child experienced a bitter taste in the mouth following the penicillin injection. Experiments with rabbits by Kagan and associates[9] demonstrated that two of three rabbits who received 1 ml. aqueous procaine penicillin intravenously, died rapidly.* Autopies on two rabbits who died two minutes after intravenous

*Autopsy reports not available, personal communication with author.

injection of 1 ml. procaine penicillin in sesame oil demonstrated fat embolism in the lungs and kidneys, pulmonary edema, and hyperemia of the liver. On the other hand, similiar clinical symptoms do appear when penicillin is administered by other routes. Thus, Leibowitz and Schwartz[10] report anaphylactic reactions from penicillin aerosol therapy and Everett[11] reports similar untoward symptoms of immediate character following instillation of penicillin into the nasal sinuses.

The types of reactions that may result from penicillin administration are too numerous to be discussed in this article, as almost any organ or organ system can be involved in such a reaction. In a general way, however, the pattern of such reactions closely simulates that following administration of heterologous serum. Thus, the symptoms may be immediate, accelerated, or delayed, the latter being a serum sickness type of reaction. Many other types of penicillin reactions may occur which, in the main, are not allergic reactions and include the Shwartzman phenomenon. The mechanism involved in the Herxheimer-like reactions, as well as the so-called ninth day erythema reactions following penicillin administration, is complicated. Possibly, some such reactions may be explained on the basis of sensitization to the antibiotic involved. In other instances, such an explanation is inadequate and the mechanism involved remains obscure.

There are certain immunologic, as well as clinical, differences between serum sickness type reactions resulting from administration of penicillin and those following heterologous serum administration. Thus, most attempts to demonstrate antibodies in the serum of patients having such reactions from penicillin have been unsuccessful, whereas such tests are frequently positive in cases of reactions following heterologous serum injections.[12] The clinical differences referred to are still more striking. When a child has had a serum sickness type of reaction from penicillin, a reinjection of this antibiotic frequently results in (1) no clinical reaction whatsoever or (2) a similar or another type of serum sickness-like reaction with the same incubation period. However, when heterologous serum is reinjected in a child, the resulting reaction is frequently of the accelerated or immediate type.

Such differences are hard to explain. Does penicillin act as a complete antigen or as a hapten? Do the ecologic or biotropic mechanisms suggested by Rostenberg and Webster[13] play a part? The answer to these questions is not clear at present.

The low incidence of penicillin anaphylaxis in children, as judged by statistical reports, is not a true measure of its frequency. It is fair to assume that one may expect a greater incidence of sensitization with continued wide use of penicillin and penicillin combinations with other drugs.

Experience has taught that history of previous symptoms resulting from penicillin contact, especially by injection, is a highly significant warning sign as to possible presence of penicillin allergy. This is especially true when such clinical reactions, even if mild, are of the immediate or accelerated variety. Had attention been given to the histories obtained on the patients in Table II,

the constitutional reactions in these children might have been avoided. On the other hand, on many occasions the histories obtained from parents are unreliable. Similarly, the symptoms following penicillin contact may be vague and not very impressive to the parent or physician. One of the authors (A. M.) has been particularly impressed with the seriousness of penicillin reactions. As a result, he has established as a routine office procedure the skin testing and periodic retesting of children, particularly children with respiratory allergy, who had previously had penicillin contact. Such a method for detecting penicillin sensitization, although uniformly negative in children who have delayed or serum sickness type of reactions, will be truly rewarding in the occasional case where potential penicillin anaphylaxis exists. In such serious immediate reactions, the skin test with penicillin is usually positive. Waldbott's[1] suggestion, of injecting subcutaneously a few drops of the penicillin to be given and waiting about a minute for possible resulting symptoms before injecting the remaining dose, is not practical in children. Furthermore, the time interval suggested for waiting is too short, as in all instances that we experienced the reactions occurred ten to twenty minutes following such injections of penicillin.

SUMMARY AND CONCLUSION

Our study of penicillin reactions in children and the use of the skin test in evaluating clinical sensitivity to this antibiotic has demonstrated the following:

1. Three hundred and ninety of a group of 391 children who had received penicillin injections on many occasions did not demonstrate skin sensitivity to same. Approximately 4 per cent of this group of children gave histories of vague symptoms only suggestive of serum sickness type of reaction following penicillin injections.

2. When another group of six children who had experienced severe constitutional reactions following administration of penicillin was similarly tested, the following results were obtained. Four of the five children who had immediate or accelerated reactions and one child who had severe, delayed, constitutional symptoms had immediate positive skin tests to penicillin. In two of three such cases, when passive transfer skin tests were performed, the tests were positive and indicated the presence in the blood of reagins to penicillin.

3. Although most of the constitutional reactions to penicillin administered occurred in those who had manifestations of atopic allergy, similar reactions occurred in nonallergic children.

4. With one exception, we were unable to find any correlation between dermal sensitivity to penicillin and that to *Penicillium notatum.*

5. An extremely high degree of clinical sensitivity to penicillin may exist in some children who have had penicillin injections previously. In one child we observed constitutional symptoms following a scratch test with penicillin of average concentration (1 drop of 5,000 units of penicillin per milliliter).

6. A negative skin test with penicillin does not rule out a potential anaphylactic-like reaction resulting from a subsequent injection of penicillin (see Case 1, Table II).

7. Anaphylactic-like reactions from penicillin administration may occur in children who have previously had no reaction from administration of this antibiotic (see Cases 2 and 3, Table II).

8. There was no dermal sensitization to procaine in the entire series of cases tested.

It is our opinion that an immediate positive skin test with penicillin in children who have had penicillin contact indicates the presence of potential clinical sensitization. When such a child, in addition, presents a history of symptoms resulting from previous penicillin administration, further injection of penicillin will likely result in a constitutional reaction. In such cases, if penicillin is the sole effective antibiotic, its oral use should be tried.

REFERENCES

1. Waldbott, George L.: Death From Penicillin, J. A. M. A. **139**: 526-527, 1949.
2. Mayer, Peter S., Mosko, M. M., and Schutz, P. J.: Penicillin Anaphylaxis, J. A. M. A. **151**: 351-353, 1953.
3. Siegal, S., Steinhardt, R. W., and Gerber, K.: Fatal and Near Fatal Penicillin Anaphylaxis, J. ALLERGY **24**: 1-10, 1953.
4. Christenson, W. N., Hedrich, G. W., and Schugman, R. F.: Fatal Anaphylactic Reaction Following Penicillin Injection, U. S. Armed Forces M. J. **4**: 249-252, 1953.
5. Feinberg, S., Feinberg, A., and Moon, C.: Penicillin Anaphylaxis, Non-Fatal and Fatal Reactions, J. A. M. A. **152**: 114-119, 1953.
6. Harpman, J. A.: Death From Penicillin, Brit. M. J. Correspondence, 392, Aug. 16, 1952.
7. Levin, S., and Moss, S. S.: Repository Penicillin Injection in Allergic Children, Ann. Allergy **9**: 471-476, 1951.
8. Collins-Williams, C., and Vincent, J.: Sensitivity Reactions to Penicillin in Children, Ann. Allergy **11**: 454-469, 1953.
9. Kagan, B. M., Nieremberg, M., Goldberg, D., and Milzer, A.: Studies of Penicillin in Pediatrics, Pediatrics **5**: 664-671, 1950.
10. Leibowitz, A., and Schwartz, E.: An unusual Reaction to Penicillin, Ann. Allergy **8**: 668, 1950.
11. Everett, R.: Anaphylactic Reaction From Local Use of Penicillin, J. A. M. A. **146**: 13-14, 1951.
12. Sherman, W. B.: Allergic Reactions to Penicillin; Panel Discussion, J. ALLERGY **24**: 396, 1953.
13. Rostenberg, A., Jr., and Webster, J.: Mechanisms of Cutaneous Drug Reactions, Especially to Antibiotics, J. A. M. A. **154**: 3-224-228, 1954.
14. Siegal, S., Steinhardt, R. W., and Gerber, K.: Fatal and Near Fatal Penicillin Anaphylaxis, J. ALLERGY **24**: 1-10, 1953.

FOLKLORE REMEDY (CANADA FLEABANE) FOR SOYBEAN DIARRHEA

"Mule Tail Tea"

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INTRODUCTION

MOST allergists probably experience moments when they would like to call upon some hidden power or magic, or consult an Indian herb doctor, in order to afford relief to their patients. A review of the lay press may strengthen such impulses when we read of the marvelous relief afforded to some allergic sufferers by cocklebur tea for asthma and alfalfa seed for arthritis,[1] fenugreek seed tea for producing mucus, jewelweed juice (touch-me-not) for relief of urticaria, and *Rauwolfia serpentina* for relief of hypertension, insomnia, and anxiety states.

Physicians, as a group, are prone to refrain from publishing or elaborating upon commonplace knowledge which they have gained through years of experience, perhaps for fear that it is not scholarly enough,[2] and will be boring to their colleagues and specialty groups. However, many unscholarly facts and experiences placed into practice prove very helpful and beneficial. A scientific worker nowadays rarely finds it possible to publish papers which have a personal touch. Some of the most interesting papers are those describing a person's own experiences, mistakes, and observations. "It is the moral obligation of every physician who has made an original scientific observation, or has formulated from his own experiences a new medical theory, to publish it for the information of his colleagues and the ultimate benefit of mankind."[9]

Such lines of reasoning have prompted the following presentation of my experience with "mule tail tea" (Canada fleabane) and the excellent results obtained for the soybean diarrheas in children who are allergic to cow's milk.

SOURCE THAT STIMULATED THE INVESTIGATION

The idea came up while I was on a fishing trip with a long-standing hunting and fishing pal, who suddenly developed a severe diarrhea while trout fishing in the wilds of the New Mexico mountains. I, being the only doctor in the crowd, was prevailed upon to remedy the man's distressing ailment, so that we could catch some fish, he being the best fisherman in the group. All of my available remedies proved of little avail. In great despair, the patient remarked that if it

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were not the fall of the year he could remedy his diarrhea by gathering leaves of a weed and making some "mule tail tea." He took an oath that he would never be caught without some of these ingredients.

This was all taken very lightly by me, but on another fishing trip a similar incident came up and my fishing buddy had his herbs on hand and quickly relieved his ailment. This interested me, and I began to look for this weed and to think about it. I had seen it grow in the sandy loam soil in my boyhood days, and had played with it, noting that it left a brown stain on my hands when the weed was green.

All allergists have had the disheartening experience of putting infants and children who are sensitive to cow's milk on soybean milk and having them receive wonderful relief from their allergic symptoms only to turn up with an aggravating diarrhea which resists Kaopectate, Amphojel, and most of the common remedies for diarrhea, to the point where the soybean milk has to be discontinued.

Most of us have heard the complaints of mothers about the foul odor of soybean milk while boiling it in an attempt to prevent the diarrhea.

Since soybean diarrhea in infants and young children allergic to cow's milk proved so baffling and defied the best modern remedies to relieve it, the idea presented itself to try this simple, and no doubt forgotten, remedy.

This was attempted with the idea that soybean diarrhea is a mechanical or chemical irritation, rather than an allergic manifestation or an infectious process.

MATERIAL USED IN THE INVESTIGATION

The remedy was used on twenty-five infants and young children who had moderate to severe diarrhea from the consumption of soybean milk (Mull-soy) after the usual methods of treating it had failed.

The first procedure in using this remedy in the soybean diarrhea was to take a pinch of the dried leaves and pour hot water over them in a teacup, similar to making a cup of common black tea and in about the same proportions. This was strained and added to the soybean formula. If this did not stop the diarrhea, then the portion was increased in size and strength and this was used as the water portion in preparing the twenty-four-hour formula. The diarrhea of all the patients was cleared up immediately by this method. When the proper portion to produce normal stools was determined, this amount was continued in the twenty-four-hour formula indefinitely. An interesting point is that, after treatment for a period of several weeks to three months, all these patients were able to tolerate soybean milk (Mull-soy) in the normal strength and formula, without having diarrhea, when the herb was removed from the formula. There were no ill effects noted from the treatment.

METHODS OF PREPARING THE HERB

The leaves of the plant were gathered in the summer (late July and early August) when the plant was in full bloom. After stripping the leaves from the plant stem, they were dried and cured by spreading them out thinly and evenly in a well-ventilated room. When well-cured and dried, they were stored in closed paper sacks with the idea of preventing molds from attacking them, and of preserving as much of the volatile oils as possible.

DESCRIPTION OF THE PLANT

A review of some of the older materia medica and pharmacology books[5-8] reveals that this herb was known and used for medicinal purposes in the early nineteenth century. It was first employed by eclectics and has been employed as a stimulant, tonic, diuretic, diaphoretic, and styptic. It was also used in diarrhea, dysentery, gravel, dropsy, and in hemorrhage of the uterus and bowels.

It was official from 1820 to 1880 and was named *Erigeron canadensis,* or Canada fleabane, and was also known as horseweed, fireweed, and colt's-tail. It is a herb of North America, being confined to Southern Canada and all of the United States except the big bend and lower tip of Texas and Florida. It grows best in sandy loam soil and waste places. It blooms in late June and July and early August. It is an annual herb 0.3 to 2.5 meters (1 to 8 feet) high. The stem branches at the top, and is hairy, furrowed, and adorned with numerous small, white flowers on the terminal panicles. The leaves are linear, lanceolate, and entire dentate, have a volatile oil content, when the herb is fresh flowering,

Fig. 1.—Canada fleabane.

of 0.2 to 0.4 per cent. When the fresh leaf is chewed, it has a slightly hot taste which is persistent and pungent. It contains a bitter principle (amaroid), tannin, and gallic acid. The oil has a specific gravity of 0.850 and contains d-limonene (C_{10} H_{16}), and terpineol. The oil is similar to oil of turpentine, but less irritating and stimulating. The recommended dose of the dried plant is 15 to 60 gr. (1 to 4 Gm.).

SUMMARY

A discarded, forgotten, folklore remedy—Canada fleabane—was used on twenty-five cases of soybean diarrhea in infants and children who were allergic to cow's milk, with good results, after the usual remedies had failed.

These patients were kept on the herb for a period ranging from a few weeks to several months, with no ill effects or side reactions.

When the diarrhea was checked and the herb was removed from the formula, they continued to tolerate soybean milk without it producing diarrhea.

CONCLUSIONS

Sometimes simple remedies, even though folklore in origin, with a scientific background and investigations, can prove very helpful in aggravating and difficult conditions.

It is well to remember the old and proved drugs and remedies and not be too prone to discard them and climb onto the proverbial bandwagon of new drugs.

REFERENCES

1. Kaufman, William H.: Alfalfa Seed Dermatitis, Report of Two Cases, J. A. M. A. **155**: 1058, 1954.
2. Campbell, Coyne H.: Practice of Clinical Psychiatry, Oklahoma M. J. **47**: 198-200, 1954.
3. Fries, Joseph H.: Dehydrated Banana in the Dietetic Management of Diarrhea of Infancy, J. Pediat. **2**: 36-37, 1950.
4. Glaser, Jerome, and Johnstone, Douglas E.: Soybean Milk as a Substitute for Mammalian Milk in Early Infancy, Ann. Allergy **10**: 433-439, 1952.
5. Culbreath, David: A Manual of Materia Medica and Pharmacology, Philadelphia, 1917, Lea & Febiger, pp. 601-602.
6. Shoemaker, John V., and Aulde, John: A Treatise on Materia Medica, Pharmacology and Therapeutics, Vol. 2, Philadelphia, 1889, F. A. Davis Company, p. 608.
7. Madaus, Gerhard: Lehrbuch der biologischen Heilmittel, Leipzig, 1938, Georg Thieme, Abteilung **1**: Heilpflanzen, Band 2, pp. 1284-1286.
8. Palter, S.: Handbook of Materia Medica, Pharmacy and Therapeutics, ed. 7, Philadelphia, 1899, The Blakiston Company, p. 231.
9. DeBakey, Selma: Suggestions on Preparation of Medical Papers, J. A. M. A. **155**: 1573, 1954.

STUDIES ON THE OPTIMUM CONCENTRATION OF GLYCERINE IN THE PREPARATION AND PRESERVATION OF RAGWEED POLLEN EXTRACT

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THE fact that glycerine protects allergenic extracts against deterioration has been known since 1917.[14, 15] The employment of concentrations up to 66⅔ per cent has been reported.[1-3, 9-11, 29, 42, 44, 51] Comparative activity of extracts was measured by direct skin test in most of the early studies. The first attempt to determine the optimum concentration of glycerine was made by Stier and Hollister,[44] in 1927, of 1 per cent weight-volume extracts of Russian thistle *(Salsola pestifer)* and timothy *(Phleum pratense)* pollens. In their studies, two-month-old undiluted extracts, containing 46 per cent glycerine and 7 per cent sodium chloride, appeared to be the most active by direct skin test (scratch). Other extracts, containing less glycerine, not only had less skin reactivity after two months, but also were contaminated. However, their original extracts were not initially processed for sterilization, such as by Seitz filtration. In 1949, Hampton, Bukantz, and Johnson[29] demonstrated the marked protective effect of 50 per cent glycerinated pollen extracts by quantitative immunologic methods, including precipitation of antiragweed rabbit sera and neutralization of human sera in passive transfer studies.

Portnoy and Sherman[38] recently reported that "Ragweed antigens preserved in an equal volume of glycerine and stored at refrigerator temperature were stable for at least one year when employed in complement fixation tests."

Preparation of highly potent extracts of some allergens, such as certain pollens, dusts, and foods, may be difficult or even impossible by the conventional methods of extraction. Therefore, some extracts, if they should contain high concentrations of glycerine, such as 50 per cent, may not be used for intradermal skin testing or injection therapy in the undiluted form because of the irritating effect of the glycerine (false positive skin tests and pain upon injection). Dilution of such extracts to nonirritating concentrations of glycerine may weaken the allergenic activity beyond practical clinical application. It was desired, therefore, to determine the minimal concentrations of glycerine capable of inhibiting deterioration of the activity of allergenic extracts.

Low ragweed *(Ambrosia elatior)* pollen was used in this study because of its extensive therapeutic use, accessibility, ease of manipulation in extraction and standardization, and adaptability to conventional means of determining allergenic activity by reagin neutralization tests.

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Hampton and associates[29] showed that an index of preservation of allergenic activity could be obtained by subjecting the 50 per cent glycerinated extracts to such procedures as storage at various temperatures, heating, and freezing, the highest index being demonstrable under conditions of ordinary refrigeration. Unglycerinated extracts, so treated, showed substantial loss of activity. The extracts used in that study contained a single concentration of glycerine that was added to a final concentration of 50 per cent after extraction was completed.

This is a presentation of studies to determine the lowest optimal concentration of glycerine necessary to maintain allergenic activity of extracts for clinical and investigational use. Extracts containing 5, 10, 20, 30, 40, and 50 per cent glycerine (by volume) were compared with unglycerinated extracts.

METHODS

A. *Extraction.*—Low ragweed pollen was defatted with ethyl ether in a Soxhlet apparatus and dried at room temperature. Seven different 20 per cent weight by volume (w/v) extracts were prepared by adding 10 gram aliquots of ether-defatted low ragweed pollen to 50 ml. aliquots of Evans-buffered saline[25] extracting fluid containing seven different concentrations of glycerine varying from 0 to 50 per cent by volume. The suspensions were placed in an automatic shaker for four hours at 4° C. and then allowed to stand at 4° C. overnight. The suspensions were filtered through Whatman #1 paper, using a Buchner funnel. Thirty-milliliter volumes of each extract were passed through a Seitz filter to sterilize, then dispensed into sterile containers and cultered for sterility.

B. *Processing of Extracts.*—Aliquots of each of the seven extracts containing 0, 5, 10, 20, 30, 40, and 50 per cent glycerine, respectively, were processed in the following manner:

1. Storage at refrigerator temperature (4° C.) for five and twelve months.
2. Storage at deep-freeze temperature (–25° C.) for five months.
3. Storage at room temperature (range, 20° C. to 40° C.) for five and twelve months.
4. Heated at 56° C. for forty-five minutes.

C. *Analytic Procedures.*—The analytic techniques employed in these studies were essentially as follows:

1. Nitrogen content was determined by methods described by Conway,[18] Ma and Zuazaga,[32] and Miller and Houghton.[33]

2. Phosphotungstic acid-precipitable (PTA) nitrogen was separated according to the method of Cooke, Stull, and associates.[19, 47, 49] One protein nitrogen unit (PNU) is equivalent to 0.00001 mg. phosphotungstic acid-precipitable nitrogen. PTA nitrogen is an acceptable index in the standardization of allergenic extracts.[19, 47, 49]

3. Reducing substances were quantitatively estimated by the methods of Somogyi[43] and Nelson.[35] The quantitative values of the reducing substances, both before and after hydrolysis with 0.5 N sulfuric acid for three hours at 100° C., were reported as glucose. The carbohydrate components were shown by paper chromatographic methods to be glucose and fructose in the unhydrolyzed sample, and glucose and arabinose in the hydrolyzed sample, as later described. Carbohydrate complexes have been studied in relation to activity.[7, 13, 26, 40, 41, 45, 46]

4. Quantitative measurements of neutralization of skin-sensitizing antibody (reagin) were performed according to the method of Cooke and coworkers.[20, 21] Briefly, the method was as follows: Equal volumes of a dilution of human serum known to sensitize passively normal human skin to ragweed pollen extract were mixed with a series of dilutions of a particular extract. Two to six hours after in vitro mixture, 0.1 ml. of each mixture was injected intracutaneously, using the skin of the back of a nonsensitive subject. Seventy-two hours later each site was tested with approximately 0.02 ml. of a known active extract (refrigerated 50 per cent glycerinated) of 1,000 PNU per milliliter concentration. The immediate wheal reactions were evaluated after about thirty minutes. The end point was considered as the site of that dilution of extract in the original mixture which failed to react upon retest.

In evaluating comparative reagin neutralization activity from the figures listed in Table II and Fig. 2, it is emphasized that comparisons were made between each concentration of glycerine within each series, rather than of absolute values of the same extracts between one series and another. The same frozen, pooled, reaginic serum was used during each series, and the same recipients were used. The values in this table represent an average of several protocols with the same series. However, except where marked changes in reactivity appear, limitations inherent in the performance of the passive transfer tests preclude comparisons of small changes in activity.

5. Determination of pH was performed on a Beckman Research G apparatus.

6. Qualitative chromatograms for identification of amino acid and carbohydrate components of the substances analyzed were run according to the methods of Consden and associates,[17] Dent,[24] and Partridge.[36, 37] Hydrolysis for bound amino acids was accomplished in a sealed tube at 110° C. for eighteen hours in 6 N hydrochloric acid. Hydrolysis for bound carbohydrates was accomplished at 100° C. for one hour in 1 N sulfuric acid, followed by neutralization with barium hydroxide until only slightly acid to phenol red indicator.

7. Sedimentation was evaluated by gross observation. No attempt was made to measure quantitatively the amount of precipitate.

RESULTS

A. *Fresh Unmodified Extracts.*—

1. ***Physical characteristics:*** There was an increase in color intensity (dark yellow) with increase in concentration of glycerine.

The time required for Seitz filtration of 30 ml. aliquots of each extract increased with increasing concentration of glycerine—ten minutes for unglycerinated to seventy-five minutes for 50 per cent glycerinated extracts.

The turbidity of the Buchner filtrate decreased with increasing concentration of glycerine. All Seitz-filtered solutions were initially free from sedimentation and turbidity, although there was slight opalescence in the unglycerinated and 5 and 10 per cent glycerinated extracts (Table I).

TABLE I. EVALUATION OF SEDIMENTATION IN EXTRACTS

EXTRACT	CONCENTRATION OF GLYCERINE (PER CENT)						
	0	5	10	20	30	40	50
Original							
Fresh extract	0	0	0	0	0	0	0
Storage at 4° C.							
Five months	++++	++++	+++	++	±	0	0
Twelve months	++++	++++	++++	+++	±	0	0
Storage at -25° C.							
Five months	++++	+++	++	0	0	0	0
Storage at 20° C to 40° C.							
Five months	++++	++++	++++	+++	+++	+++	++
Twelve months	++++	++++	++++	++++	++++	+++	++
Heated 56° C. for 45 minutes							
Fresh extract	++++	++++	+++	++	0	0	0

2. *pH:* The pH of each of the seven freshly prepared extracts was approximately 5.4 (Table V).

3. *PTA nitrogen and total nitrogen:* The quantitative nitrogen (total and phosphotungstic acid-precipitable) and carbohydrate (free, bound, and total) values of each of the extracts were approximately equal, regardless of the concentration of glycerine (Tables III and IV, Figs. 3 and 4).

The PTA nitrogen content of the extracts averaged 0.84 mg. per milliliter, with the exception of the 5 per cent glycerinated extract which contained about 10,000 fewer protein nitrogen units (and proportionally lower carbohydrate values). This extract was prepared in the ratio of 5 grams to 25 ml. of extracting fluid, instead of 10 grams to 50 ml. as were the other six extracts. This smaller volume of extract may have resulted in a proportionally greater absorption of PTA nitrogen and carbohydrate substances on the Seitz filter pad during sterilization.

The total nitrogen values of the seven freshly prepared extracts averaged 1.99 mg. per milliliter. The total nitrogen/PTA nitrogen ratio of the extracts was 2.4.

4. *Reducing substances:* Analysis of the original freshly prepared extracts revealed that 40 to 50 per cent of the total reducing substances in the extracts were in the unbound state (Table IV, Fig. 4). The 40 and 50 per cent glycerinated extracts contained the greatest amount (60 per cent) of bound carbohydrate.

5. *Chromatographic analysis:* Chromatograms (two-dimensional, phenol-lutidine) for ninhydrin-reacting components of unhydrolyzed and hydrolyzed

samples of each of the seven extracts were qualitatively similar to the findings reported by Dankner and associates.[23] It was noted that the increase in concentration of glycerine seemed to result in a decrease in the number, intensity, and separation of ninhydrin-reacting areas of chromatographed samples. Hydrolysis in sealed ampules in 6 N hydrochloric acid at 110° C. for eighteen hours apparently failed to hydrolyze completely those samples containing glycerine above 10 per cent concentration. Glycerine was added in a concentration of 50 per cent to artificial mixtures of known quantities of amino acids and to unglycerinated ragweed extracts. These mixtures were chromatographed two-dimensionally in phenol and lutidine. There was marked streaking, smearing, and consolidation of all ninhydrin-reacting areas after chromatography of the 50 per cent glycerinated samples, both hydrolyzed and unhydrolyzed. Identification and evaluation of these areas were impossible. Clear-cut differentiation was obtained with the corresponding unglycerinated controls.

No attempt was made to remove the glycerine from the pollen extracts before chromatographing.

TABLE II. NEUTRALIZATION OF SKIN-SENSITIZING ANTIBODY*

EXTRACT	CONCENTRATION OF GLYCERINE (PER CENT)						
	0	5	10	20	30	40	50
Original							
Fresh extract	50	50	75	50	75	50	50
Storage at 4° C.							
Five months	750	500	250	150	150	100	100
Twelve months	500	250	150	100	150	150	100
Storage at -25° C.							
Five months	500	250	150	100	75	75	75
Storage at 20° C. to 40° C.							
Five months	>3,000	>3,000	>3,000	>3,000	2,000	1,500	750
Twelve months	>3,000	>3,000	>3,000	>3,000	>3,000	>3,000	750
Heated 56° C. for 45 minutes							
Fresh extract	>3,000	>3,000	>3,000	>3,000	750	250	250

*End point = Lowest concentration of extract (in PNU per milliliter) added to an equal volume of serum dilution which resulted in a negative reaction upon retest with refrigerated 50 per cent glycerinated extract, diluted to 1,000 PNU per milliliter.

One-dimensional chromatograms in phenol for identification of carbohydrate components of each extract revealed glucose and fructose before hydrolysis and glucose and arabinose after hydrolysis. These findings were similar to those noted by Frankel and co-workers.[26] Increasing the concentration of glycerine resulted in fainter aniline hydrogen phthalate[37] reacting areas in the position of arabinose on chromatograms of unhydrolyzed samples. Chromatograms of artificial mixtures of arabinose and comparable concentrations of glycerine indicated that the presence of glycerine did not lessen the total amount of color development but increased the area of the spot. These results lead to the impression that glycerine protects against dissociation of the bound carbohydrate complexes.

CONCENTRATION OF EXTRACT (PNU/ML.) ADDED TO EQUAL VOLUMES OF SERUM (#53-G, 1-5)	CONCENTRATION OF GLYCERINE (PER CENT)						
	0	5	10	20	30	40	50
1	+++	+++	+++	+++	+++	+++	+++
5	++	++	+++	++	+++	+++	++
10	++	++	++	++	++	++	+
25	+	+	+	+	+	+	+
50	0	0	±	0	±	±	±
75	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0
200	0	0	0	0	0	0	0
400	0	0	0	0	0	0	0
Diluent	++++	++++	++++	++++	++++	++++	++++

Fig. 1.—Neutralization of skin-sensitizing antibody by fresh extracts of ragweed pollen containing different concentrations of glycerine. End point: Lowest concentration of extract (in PNU per milliliter) added to an equal volume of serum dilution which resulted in a negative reaction upon retest with refrigerated 50 per cent glycerinated extract, diluted to 1,000 PNU per milliliter.

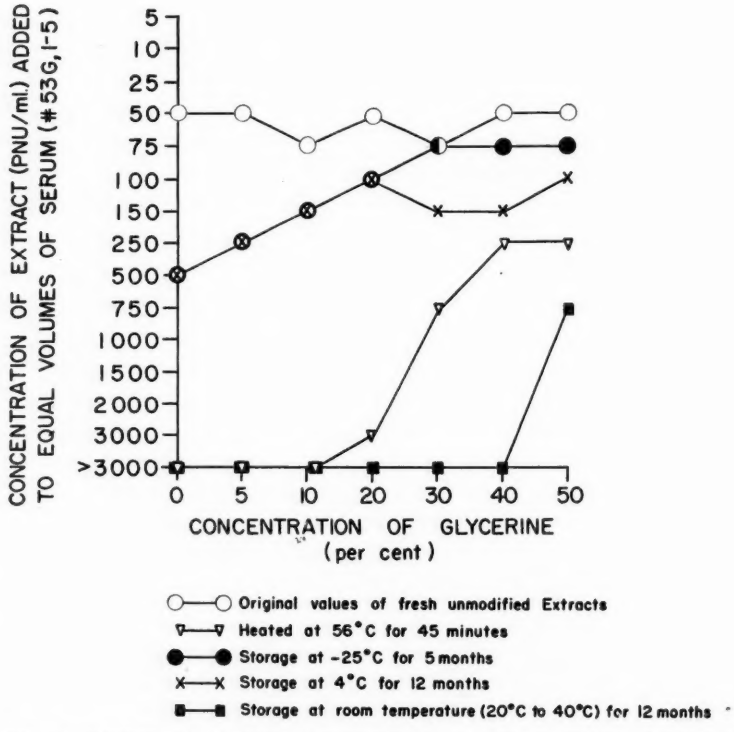

Fig. 2.—Effect of storage at different temperatures on neutralization of skin-sensitizing antibody of ragweed pollen extracts containing different concentrations of glycerine.

6. *Neutralization of skin-sensitizing antibody (reagin):* The reagin neutralization activity of the seven extracts, when diluted to equal PTA nitrogen concentration, was uniformly linear (Table II, Fig. 2). The end point of each extract was approximately 50 units, as illustrated in Fig. 1.

B. *Storage at Refrigerator Temperature (4° C.) for Five and Twelve Months.* —

1. *Physical characteristics:* Sedimentation appeared in quantities inversely related to the concentration of glycerine (Table I). Extracts containing glycerine in the concentration of 30 per cent or greater contained no precipitate. The amorphous, dull yellow sediment formed in refrigerated, unglycerinated extracts of ragweed pollen was found, in other studies,[30] to be a protein or polypeptide complex containing flavonoid and reducing substances. It was found to be allergenically active by direct skin tests and by reagin neutralization tests. The possibility of contaminating active substances was not excluded. Stull and his associates[47, 48] and Moore and his associates[34] have reported studies on such a sediment appearing spontaneously in ragweed pollen extracts.

The preceding and following comments on sedimentation during various conditions of storage concern undiluted extracts containing the same original amount of activity, protein nitrogen, and carbohydrate content but different concentrations of glycerine. An incidental observation was the formation of sediment which occurred regularly when these and other[30] clear, 50 per cent glycerinated extracts of 20 per cent weight by volume concentration (containing 80,000 to 120,000 PNU per milliliter) of ragweed pollen were diluted 1 to 5 with unglycerinated buffer. This sediment appeared about seven days after dilution. No sediment appeared in the weaker dilutions of 1:10, 1:20, 1:40, 1:100, and 1:1,000.

The sediment of this 1 to 5 dilution (containing 10 per cent glycerine) of the ragweed extract was packed by centrifugation. The glycerine content of the supernate was restored to the concentration of 50 per cent while keeping the volume equal to the original volume of the sample of extract centrifugalized. The mixture was then stirred and shaken to suspend the sediment. A control sample without glycerine was similarly processed. Centrifugation, after standing for eighteen hours at 4° C., resulted in no sediment in the 50 per cent glycerinated sample and little or no diminution of amount of precipitate in the unglycerinated sample. Furthermore, addition of buffered saline to the 1 to 5 dilution, containing sediment, to a final dilution of 1 to 10 and 1 to 20 resulted in a sediment which went into solution when the glycerine concentration was restored to 50 per cent or when the extract was diluted further with buffered saline.

The appearance of sedimentation in relation to pH of extracting fluid and to final pH of the extract, both adjusted and unadjusted, was studied briefly. The results are summarized in Table VI. Extraction of low ragweed pollen in a 20 per cent weight by volume ratio with Evans-buffered saline, plain and 50 per cent glycerinated, of pH 9 resulted in a final extract of pH 5.7, only 0.2

pH higher than extraction with neutral fluid of pH 7.0 resulting in pH of 5.5. This buffer required alkali (sodium hydroxide) to pH 11.5 to result in a final extract of pH 7.0. The extract reaches its pH apparently after fifteen minutes of extraction, as there was no change in pH after standing overnight at 4° C. Obviously, the buffering action of pollen cell constituents was quite strong. The extracts, both plain and glycerinated, prepared with fluids above neutral pH (9.0 and 11.5) were more opalescent than when prepared with neutral extractants. Adjusting the pH, with 2.5 N sodium hydroxide, of the acidic (pH 5.5 and 5.7) plain and glycerinated extracts (which had been prepared with fluids of pH 7.0 and 9.0, respectively) to pH 7.0 resulted in the immediate formation of moderate sediment and in the darkening of the color of the supernatant fluids.

TABLE III. PHOSPHOTUNGSTIC ACID-PRECIPITABLE NITROGEN*

EXTRACT	CONCENTRATION OF GLYCERINE (PER CENT)						
	0	5	10	20	30	40	50
Original							
Fresh extract	0.81	0.74	0.84	0.85	0.86	0.81	0.88
Storage at 4° C.							
Five months	0.66 (81%)†	0.60 (81%)	0.67 (80%)	0.69 (81%)	0.70 (82%)	0.64 (79%)	0.75 (85%)
Twelve months	0.66 (81%)	0.60 (81%)	0.64 (76%)	0.70 (82%)	0.69 (81%)	0.65 (80%)	0.75 (85%)
Storage at -25° C.							
Five months	0.65 (81%)	0.65 (88%)	0.70 (84%)	0.79 (93%)	0.78 (91%)	0.73 (90%)	0.78 (89%)
Storage at 20° C. to 40° C.							
Five months	0.65 (81%)	0.63 (85%)	0.65 (77%)	0.65 (77%)	0.67 (78%)	0.69 (85%)	0.72 (82%)
Twelve months	0.60 (74%)	QNS	0.60 (72%)	0.57 (67%)	0.59 (69%)	0.65 (80%)	0.73 (83%)
Heated at 56° C. for 45 minutes							
Fresh extract	0.78 (97%)	0.74 (100%)	0.83 (99%)	0.83 (98%)	0.82 (96%)	0.79 (98%)	0.86 (98%)

*Milligrams per milliliter.
†Per cent of original value of fresh extract.

Adjusting the pH of neutral (pH 7.0) plain and glycerinated extracts (prepared with fluids of pH 11.5) to 5.5 resulted in immediate opalescence and the formation of small amounts of fine, glistening precipitate. No marked differences in PTA nitrogen content were found in analysis of fresh extracts, plain or glycerinated, of any of the three unadjusted pH values (5.5, 5.7, and 7.2). The use of the alkaline (pH 11.5) extracting fluid to produce extracts of pH 7.2 resulted in slightly lower concentration of total or bound carbohydrate compared with the more acidic extracts. The quantity of free carbohydrate was about equal to that found in the acidic extracts (Table VI).

Adjusting neutral extracts to pH 5.5 resulted in much less sediment (initially and on standing) and of a different color and character than adjusting acidic extracts to neutral, which resulted in heavy initial sedimentation followed by further delayed precipitation (Table VI).

2. *pH:* There was only a slight fall in pH (average 0.1) after five months, regardless of concentration of glycerine. At twelve months, however, a greater (though still only minor) change in pH was evident, the drop ranging from 0.06 to 0.30. The 50 per cent glycerinated showed the least change (Table V).

The undiluted extracts containing 84,000 PNU per milliliter and dilutions of 20,000 and 10,000 PNU per milliliter showed a slight drop in pH, while the more dilute solutions of 1,000, 100, and 10 PNU per milliliter showed a slight increase in pH. Criep and his associates[22] reported increases in pH with aging of dilute extracts which contained 0.01 mg. total nitrogen per milliliter. Phosphate buffer was used as diluent in both studies.

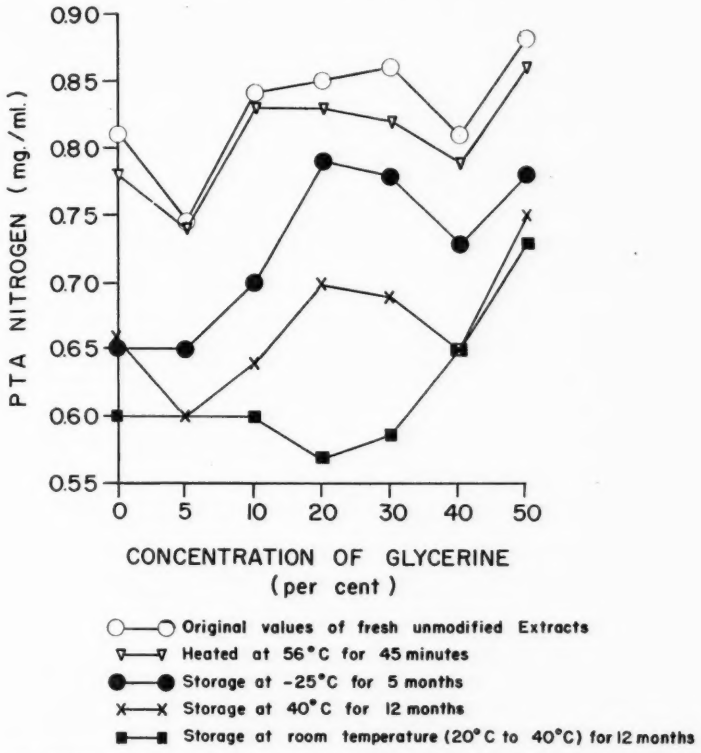

Fig. 3.—Effect of storage at different temperatures on phosphotungstic acid precipitable nitrogen content of ragweed pollen extracts containing different concentrations of glycerine.

3. *PTA nitrogen:* The loss in PTA nitrogen (Table III, Fig. 3) of all seven extracts at five months was about 20 per cent. The 50 per cent glycerinated lost only 15 per cent. Analysis at twelve months resulted in values which approximated those at five months.

4. *Reducing substances:* There was an increasing protective effect with increasing concentrations of glycerine (Table IV, Fig. 4) as reflected by the range of free carbohydrate from 89 per cent to 44 per cent at six months. There was a uniform loss, although slight, of bound carbohydrate after twelve

TABLE IV. CONCENTRATION OF REDUCING SUBSTANCES*

EXTRACT	CONCENTRATION OF GLYCERINE† (PER CENT)																				
	0			5			10			20			30			40			50		
	T	F	B	T	F	B	T	F	B	T	F	B	T	F	B	T	F	B	T	F	B
Original																					
Fresh extract	14.0	6.7 (48%)‡	7.3	12.5	6.5 (52%)	6.0	13.2	6.3 (48%)	6.9	14.9	7.1 (48%)	7.8	13.0	6.0 (46%)	7.0	14.6	5.8 (40%)	8.5	14.3	5.8 (41%)	8.5
Storage at 4° C.																					
Five months	11.7	10.4 (89%)	1.3	12.4	9.6 (79%)	2.8	12.1	7.9 (65%)	7.3	14.6	7.3 (50%)	7.3	13.3	7.3 (55%)	6.0	14.9	6.7 (45%)	8.2	14.4	6.4 (44%)	8.0
Twelve months	13.1	12.2 (93%)	2.9	11.0	8.7 (79%)	2.3	13.4	8.9 (66%)	4.5	14.1	9.3 (66%)	4.8	14.5	8.2 (57%)	6.3	14.6	6.7 (46%)	7.9	13.6	6.4 (47%)	7.2
Storage at -25° C.																					
Five months	12.6	7.6 (61%)	5.0	10.7	6.1 (57%)	4.6	12.3	6.6 (53%)	5.7	12.3	6.9 (56%)	5.4	12.1	6.9 (57%)	5.2	12.1	6.7 (55%)	5.4	13.5	7.2 (53%)	6.3
Storage at 20° C. to 40° C.																					
Five months	12.7	13.0 (100%)	0	11.5	11.6 (100%)	0	12.0	12.0 (100%)	0	10.3	11.3 (100%)	0	12.0	8.7 (73%)	3.3	12.1	8.6 (71%)	3.5	13.6	7.8 (56%)	5.8
Twelve months	12.2	12.2 (100%)	0	12.6	12.7 (100%)	0	12.7	12.9 (100%)	0.8	12.2	12.1 (99%)	0.1	13.4	10.6 (79%)	2.8	13.0	9.2 (71%)	3.8	13.4	8.1 (61%)	7.1
Heated at 56° C. for 45 minutes																					
Fresh extract	14.1	6.8 (48%)	7.3	12.6	6.6 (52%)	6.0	13.1	6.2 (47%)	6.9	14.7	7.0 (48%)	7.7	12.9	6.0 (47%)	6.9	14.8	5.8 (39%)	8.9	14.1	5.6 (40%)	8.5

*As glucose, milligrams per milliliter.

†T, Total; F, free; B, bound.

‡Per cent of total carbohydrate which was unbound (free).

months. The 40 and 50 per cent glycerinated extracts showed the greatest protection with only a 6 to 10 per cent increase in free sugar as compared with a 45 per cent increase in the unglycerinated.

5. *Neutralization of skin-sensitizing antibody (reagin):* The loss in reagin neutralization activity of extracts stored at 4° C. was less evident than that of heated extracts or those stored at room temperature (Table II, Fig. 2). However, there was quite a loss of this activity in the unglycerinated extracts. There was evidence of protection against such loss even by a concentration of glycerine as low as 20 per cent. The values at twelve months approximated those at five months (Table II).

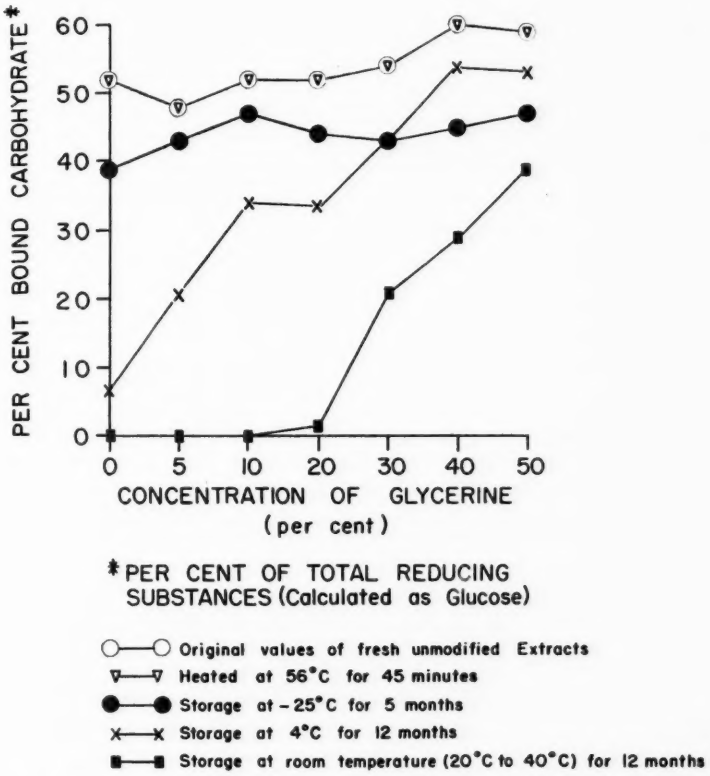

Fig. 4.—Effect of storage at different temperatures on bound carbohydrate content of ragweed pollen extracts containing different concentrations of glycerine.

C. *Storage at Deep-Freeze Temperature (–25° C.) for Five Months.*—

1. *Physical characteristics:* All extracts, including 50 per cent glycerinated, were completely frozen at time of removal from the deep freeze. They melted in order of decreasing glycerine content.

Sediment (brownish yellow) was found in the unglycerinated and the 5 and 10 per cent glycerinated extracts. No attempt was made to characterize this sediment (Table I).

2. *pH:* There was only a slight rise of 0.02 to 0.2 pH noted in the unglycerinated extracts and those of low concentrations of glycerine (Table V).

3. *PTA nitrogen:* The change in PTA nitrogen was unrelated to glycerine content. Concentrations of glycerine of 10 per cent and greater retained an average of 90 per cent of the original PTA nitrogen content (Table III).

4. *Reducing substances:* There was a 6 to 16 per cent increase in the free carbohydrate of all the extracts (Table IV and Fig. 4). The amount of change was unrelated to the concentration of glycerine.

TABLE V. DETERMINATION OF pH

EXTRACT	CONCENTRATION OF GLYCERINE (PER CENT)							
	0	5	10	20	30	40	50	EXTRACTING FLUID 0 AND 50
Original								
Fresh extract	5.40	5.40	5.38	5.38	5.40	5.40	5.35	6.95
Storage at 4° C.								
Five months	5.30 (−0.10)	5.30 (−0.10)	5.35 (−0.05)	5.25 (−0.13)	5.30 (−0.10)	5.30 (−0.10)	5.32 (−0.03)	7.00 (+0.05)
Twelve months	5.10 (−0.00)	5.20 (−0.20)	5.15 (−0.23)	5.12 (−0.26)	5.20 (−0.20)	5.27 (−0.13)	5.29 (−0.06)	7.00 (+0.05)
Storage at −25° C.								
Five months	5.60 (+0.20)	QNS	5.48 (+0.10)	5.42 (+0.04)	5.45 (+0.05)	5.42 (+0.02)	5.42 (+0.07)	7.00 (+0.05)
Storage at 20° C to 40° C.								
Five months	5.00 (−0.40)	5.00 (−0.40)	5.00 (−0.38)	5.00 (−0.38	5.02 (−0.38)	5.05 (−0.35)	5.20 (−0.15)	7.00 (+0.05)
Twelve months	5.00 (−0.40)	QNS	5.00 (−0.38)	4.95 (−0.43)	4.80 (−0.60)	4.97 (−0.43)	5.01 (−0.34)	7.00 (+0.05)
Heated at 56° C. for 45 minutes								
Fresh extract	5.41 (+0.01)	5.40 (0.0)	5.39 (+0.01)	5.39 (+0.01)	5.42 (+0.02)	5.40 (0.0)	5.35 (0.0)	7.00 (+0.05)

Figures in parentheses indicate change from original value of fresh unmodified extract.

5. *Neutralization of skin-sensitizing antibody (reagin):* There was a slight to moderate loss in reagin neutralization activity of the unglycerinated and slightly glycerinated extracts (Table II and Fig. 2). Thirty and 40 per cent glycerine offered good protection and almost equalled that of the 50 per cent glycerinated extract. The activity of these extracts was comparable to the refrigerated extracts.

D. *Storage at Room Temperature (Range, 20° C. to 40° C.) for Five and Twelve Months.* —

1. *Physical characteristics:* All extracts contained sediment (Table I). The sediment of the respective extracts did not appear the same, varying from amorphous, chocolate brown to granular yellow in different combinations. No attempt was made to characterize this sediment. Cultures for sterility at five and twelve months resulted in no growth of organisms.

2. *pH:* Of the four types of processing studied, storage at room temperature resulted in the greatest changes in pH (Table V). The range of drop in

pH was 0.15 to 0.40 and 0.34 to 0.60 at five and twelve months, respectively. The amount of drop seemed independent of the concentration of glycerine, with the exception that the 50 per cent glycerinated showed the least change at both periods of study.

3. *PTA nitrogen:* PTA nitrogen values showed a general loss of about 15 and 20 per cent at six months (Table III and Fig. 3). At twelve months, however, the range of losses increased to 17 and 33 per cent. The 50 per cent glycerinated extract retained 83 per cent of its original PTA nitrogen.

Stull and his associates[48] noted that incubation of fresh extracts of timothy and ragweed pollen for ten days at 37° C. caused a loss in PTA nitrogen as great as that which occurred in extracts during one year at 7° C.

4. *Reducing substances:* There was a marked loss in bound carbohydrate (Table IV and Fig. 4). The unglycerinated and the 5, 10, and 20 per cent glycerinated extracts lost 100 per cent of their bound carbohydrate. The 30

CONCENTRATION OF EXTRACT (PNU/ML.) ADDED TO EQUAL VOLUMES OF SERUM (#53-G, 1-5)	CONCENTRATION OF GLYCERINE (PER CENT)							
	HEATED							UN-HEATED
	0	5	10	20	30	40	50	50
1							++++	++++
5					+++	+++	+++	+++
10					+++	+++	+++	++
25					+++	+++	+++	+
50	++++	++++	++++	++++	+++	+++	+++	±
75	++++	++++	++++	++++	+++	++	++	0
100	++++	++++	++++	++++	+++	+	+	0
150	++++	++++	++++	++++	++	+	+	0
250	++++	++++	++++	++++	+	0	0	0
500	++++	++++	+++	+++	+	0	0	0
750	++++	+++	+++	+++	0			
1,000	++++	+++	+++	++				
1,500	++++	+++	++	++				
2,000	++++	++	++	++				
3,000	+++	++	++	++				
Diluent	++++	++++	++++	++++	++++	++++	++++	++++

Fig. 5.—Neutralization of skin-sensitizing antibody by fresh ragweed extracts after heating at 56° C. for forty-five minutes. End point: Lowest concentration of extract (in PNU per milliliter) added to an equal volume of serum dilution which resulted in a negative reaction upon retest with refrigerated 50 per cent glycerinated extract, diluted to 1,000 PNU per milliliter.

per cent glycerinated extract indicated some protection, its free sugar increasing from 46 per cent to 73 per cent at five months and to 79 per cent at twelve months. However, the 40 and 50 per cent glycerinated showed the least loss of bound sugar; their free carbohydrate values, which average 40 per cent originally, became 45 per cent at six months and 46 per cent at twelve months.

5. *Neutralization of skin-sensitizing antibody (reagin):* The loss in reagin neutralization activity was marked in the extracts with low concentrations of glycerine. Thirty per cent glycerine showed slight protection, 40 per cent still greater, and 50 per cent the greatest protection at five months. At twelve months, only the 50 per cent glycerine afforded protection (Table II and Fig. 2).

TABLE VI. EFFECT OF pH UPON OCCURRENCE OF SEDIMENTATION

	pH EXTRACTING FLUID	pH EXTRACT	PTA NITROGEN (MG./ML.)	REDUCING SUBSTANCES (MG./ML.)* F	REDUCING SUBSTANCES (MG./ML.)* T	REDUCING SUBSTANCES (MG./ML.)* B†	SEDIMENTATION 1 DAY	SEDIMENTATION 1 WEEKS	SEDIMENTATION 4 WEEKS	SEDIMENTATION 26 WEEKS	pH ADJUSTED TO	SEDIMENTATION 1 DAY	SEDIMENTATION 1 WEEKS	SEDIMENTATION 4 WEEKS	SEDIMENTATION 26 WEEKS
Plain	7.0	5.5	0.89	5.2	11.4	6.2	0	+	++	+++	7.0	+++	+++	++++	++++
	9.0	5.7	0.89	5.7	12.2	6.5	+	+	++	+++	7.0	+++	++++	++++	++++
	11.5	7.2	0.93	5.0	9.7	4.7	+	+	++	+++	5.5	+	+	+	+
50 per cent Glycerinated	7.0	5.5	0.86	5.8	13.9	8.1	0	0	0	0	7.0	+++	++++	++++	++++
	9.0	5.7	0.78	5.0	11.8	6.8	0	0	0	0	7.0	+	+++	+++	+++
	11.5	7.2	0.83	5.7	10.2	4.5	0	0	0	0	5.5	+	+	+	+

*As glucose.

†T, Total; F, free; B, bound.

E. *Heating at 56° C. for 45 Minutes.*—

1. *Physical characteristics:* There was increasing sedimentation of amorphous, dull yellow precipitate with decreasing concentration of glycerine (Table I).

2. *pH:* The pH of all extracts was unaffected by heating (Table V).

3. *PTA nitrogen:* There was no change in PTA nitrogen content, regardless of the concentration of glycerine (Table III and Fig. 3).

4. *Reducing substances:* Heating produced no effect upon the values of free, bound, and total carbohydrate of the seven extracts (Table IV and Fig. 4).

5. *Neutralization of skin-sensitizing antibody (reagin):* The changes in the reagin-neutralizing activity of the seven extracts as the result of heating were outstanding (Table II and Figs. 2 and 5). The loss of activity was inversely proportional to the concentration of glycerine. Even the 50 per cent glycerinated extract showed a diminution of activity compared with its unheated control.

DISCUSSION

The present investigation clearly demonstrates that glycerine in concentration of 30 to 50 per cent inhibited deterioration of the allergenic activity of ragweed pollen extracts, prepared in the concentration of 20 grams per 100 ml. of extracting fluid. Therefore, such extracts may be prepared and stored in the refrigerator for possibly indefinite periods of time without fear of loss of potency. The great value of this feature in research, as well as in clinical medicine, is obvious.

Observations during a separate but simultaneous study[30] on the maximum weight by volume extractibility of pollen, using unglycerinated and 50 per cent glycerinated ragweed and timothy pollen extracts, indicated the absolute necessity of glycerine in preparing 10 per cent to 50 per cent pollen extracts (10 to 50 grams pollen per 100 ml. extracting fluid). All twelve 50 per cent glycerinated extracts (1 to 50 per cent pollen extraction) remained clear and without sediment, while the unglycerinated extracts showed initial turbidity and subsequent marked sedimentation.

This particular study afforded evaluation of changes in quantities of bound carbohydrate in relation to concentration of glycerine, as extracts of ragweed pollen contain bound reducing substances. However, extracts of certain other pollens may not contain bound carbohydrate, which could be measured by an increase in reducing substances after hydrolysis. Analysis of timothy pollen extracts shows no change or a slight loss in reducing power after hydrolysis.[30] Similar findings have been made by Stull, Cooke, and Chobot[46, 50] with extracts of timothy and cattail pollen.

Studies by Hampton and his associates,[29] with plain and 50 per cent glycerinated extracts, involved the use of weight by volume and PTA nitrogen concentrations of 2.5 per cent and 10,000 units per ml., respectively, whereas those used in the present studies were 20 per cent and 85,000 units

per milliliter. The earlier studies also covered a period of storage of twelve months, and the marked protective effect of 50 per cent glycerine was most evident. The deteriorating effects of storage, heating, and freezing in both series were quite similar.

As early as 1917, Clock[14] noted that when pollens were extracted in 66⅔ per cent glycerine and 33⅓ per cent saturated sodium chloride, the extracts showed no deterioration after fourteen months at 5° C., as determined by complement fixation tests. Recently Portnoy and Sherman,[38] using precise quantitative techniques of measuring changes in complement fixation activity, noted that ragweed antigens in 50 per cent glycerine retained their activity for at least one year at refrigerator temperature.

In 1949, this laboratory[29] reported a greater difference in neutralizing activity between frozen plain extracts and frozen glycerinated extracts than was noted in the present study. This difference might be accounted for by the facts that: (1) different reaginic sera were used; (2) in the first study, the sediment which formed in the frozen, thawed, plain extract, was removed by centrifugation before analysis for PTA nitrogen content and for activity; and (3) the extracts (85,000 PNU per milliliter) in the present study were eight times more concentrated and may have resisted the deleterious effects of freeze-thawing better than the more dilute extracts (10,000 PNU per milliliter) used in the first study. Black,[8] in 1925, reported that when pollen extracts were frozen solid for six days there was no loss in activity as measured by nasal reactivity. Bukantz and associates[12] studied the effect of freeze-thawing on the capacity of ragweed extract to precipitate antiragweed rabbit serum, and Hampton and his co-workers[29] showed the protection of 50 per cent glycerine in maintaining the precipitating activity of the extracts.

In most of the earlier studies, the effect of heat on pollen extracts was evaluated in terms of skin reactivity,[16, 27, 28, 31] nasal membrane reactivity,[8] capacity to exhaust passively sensitized sites,[46] and complement fixation activity.[5, 6] Later studies, employing quantitative estimation of neutralization of skin-sensitizing antibody,[4, 29, 48] reported marked diminution of activity by heat. The protective effect of glycerine against deterioration by heat was noted as early as 1924 by Armstrong and Harrison.[5, 6] This beneficial effect of glycerine was also studied in this laboratory when plain and 50 per cent glycerinated extracts were heated at 56° C. for thirty minutes.[29] In agreement with the present study, Arbesman and Eagle[4] reported conclusions that deterioration produced by heat was not accompanied by significant changes in protein nitrogen content. Earlier studies from this laboratory[29] resulted in little or no decrease of PTA nitrogen of the plain extracts after heating to 56° C. for thirty minutes. Stull and associates[48] reported that heating to 70° C. caused a precipitation and loss of protein nitrogen (after removal of the precipitate by centrifugation) in ragweed extracts, but none in timothy extracts. The heating of dry pollen before extraction, as studied by Rappaport[39] and by Stull and co-workers,[46] was not part of this investigation.

Hampton and his co-workers[29] noted a loss in precipitating activity, as well as in reagin neutralization and PTA nitrogen, when unglycerinated and

50 per cent glycerinated extracts were stored at room temperature. In 1924 Armstrong and Harrison[5] reported no apparent loss in complement-fixating power after 144 days at room temperature.

In concluding the present study, it is observed that 20 per cent glycerine does not inhibit loss in PTA nitrogen and bound carbohydrate, even though this concentration of glycerine protects against loss of neutralization activity when ragweed extracts are stored in the refrigerator for one year. Since there was a marked difference in protection of neutralization capacity between the 40 and 50 per cent glycerinated extracts when stored at room temperature, storage in the refrigerator for more than one year might result in loss of neutralization activity in concentration of glycerine of less than 50 per cent.

SUMMARY

1. Extracts of low ragweed pollen, prepared in the concentration of 20 grams per 100 ml. of extracting fluid which contained 0, 5, 10, 20, 30, 40, and 50 per cent glycerine by volume, were observed after storage for five and twelve months at refrigerator (4° C.), deep-freeze (–25° C.), and room temperatures (20° C. to 40° C.), as well as heating at 56° C. for forty-five minutes.

2. Each extract was evaluated in terms of appearance of sediment, changes in concentration of PTA nitrogen and reducing substances, and loss of activity in neutralization of skin-sensitizing antibody.

3. The higher the concentration of glycerine, the greater was the protective effect against loss of reagin-neutralization activity at the various temperatures studied.

4. There was essentially no change in PTA nitrogen after heating, regardless of the concentration of glycerine. There was, however, an appreciable loss in PTA nitrogen of the stored extracts, most pronounced in those extracts containing smaller concentrations of glycerine.

5. The loss in bound carbohydrate varied under the different conditions of temperature. There was no change in any extract after heating at 56° C. for forty-five minutes. However, at room temperature, glycerine in the higher concentration showed a protective effect not evident in the lower concentrations.

6. An important observation was the clarity of extracts of high glycerine content in comparison with the sedimentation in extracts containing either no glycerine or glycerine of low concentration.

7. Whereas 30 and 40 per cent glycerine afforded significant protection against deterioration, the greatest protection was observed at the 50 per cent concentration.

REFERENCES

1. Alexander, H. L.: Treatment of Hay Fever by Specialists in the United States and Canada; Report of an Inquiry, J. ALLERGY **4**: 169, 1933.
2. Alles, G. A., and Lamson, R. W.: Preparation and Purification of Pollen Extracts, J. Immunol. **14**: 181, 1927.
3. Alles, G. A., Piness, G., and Miller, H.: Preparation and Stability of Food Allergen Extracts, J. ALLERGY **5**: 5, 1933.

4. Arbesman, C. E., and Eagle, H.: Thermolability of Ragweed Pollen Extract and Its Corresponding Reagin, J. ALLERGY **11**: 18, 1939.
5. Armstrong, C., and Harrison, W. T.: A Study in Ragweed Pollen Extracts for Use in Treatment of Ragweed Pollen Hypersensitiveness, Pub. Health Rep. **39**: 2422, 1924.
6. Armstrong, C., and Harrison, W. T.: Standardization of Pollen Extracts by Complement-Fixation Test, Pub. Health Rep. **40**: 1466, 1925.
7. Black, J. H.: A Soluble Specific Carbohydrate of Ragweed Pollen, J. ALLERGY **2**: 161, 1931.
8. Black, J. H.: Pollen Sensitization. A Preliminary Report, J. Lab. & Clin. Med. **10**: 378, 1925.
9. Brown, A.: The Use of Glycerolated Pollen Extracts in the Treatment of Hay Fever, J. ALLERGY **4**: 468, 1933.
10. Brown, G. T.: Maximum Dosage Pollen Therapy, J. ALLERGY **3**: 180, 1932.
11. Brown, G. T.: Further Experiences With Maximum Dosage Pollen Therapy, J. ALLERGY **6**: 86, 1934.
12. Bukantz, S. C., Johnson, M. C., and Hampton, S. F.: Quantitative Immunologic Studies With Allergens. I. Colorimetric Absolute Methods in the Analysis of Factors Influencing Precipitation of Ragweed Pollen Extracts and Homologous Rabbit Antisera, J. ALLERGY **20**: 1, 1949.
13. Caulfeild, A. H. W.: Brown, M. H., and Waters, E. T.: Experiments to Determine Whether the Allergically Active Substance in Ragweed Pollen Extract is a Single Entity or Multiple, J. ALLERGY **7**: 1, 1935.
14. Clock, R. O.: A Stable Pollen Antigen, J. Infect. Dis. **21**: 387, 1917.
15. Clock, R. O.: Preservation of Anti-Sheep Hemolytic Amboceptor in Glycerol, J. Infect. Dis. **21**: 404, 1917.
16. Coca, A. F., Walzer, M., and Thommen, A. A.: Asthma and Hay Fever in Theory and Practice, Springfield, Ill., 1931, Charles C Thomas, p. 737.
17. Consden, R., Gordon, A. H., and Martin, A. J. P.: Qualitative Analysis of Proteins. A Partition Chromatographic Method Using Paper, Biochem. J. **38**: 224, 1944.
18. Conway, E. J.: Micro Diffusion Analysis and Volumetric Error, London, 1947, Crosby Lockwood & Son, Ltd., p. 85.
19. Cooke, R. A., and Stull, A.: Preparation and Standardization of Pollen Extracts for Treatment of Hay Fever, J. ALLERGY **4**: 87, 1932.
20. Cooke, R. A., Stull, A., Hebald, S., and Barnard, J. H.: Clinical and Serologic Study of Relationship of Giant (Ambrosia trifida) and Low (Ambrosia artemisiaefolia) Ragweed Pollen, J. ALLERGY **6**: 311, 1935.
21. Cooke, R. A., Barnard, J. H., Hebald, S., and Stull, A.: Serological Evidence of Immunity With Coexisting Sensitization in a Type of Human Allergy (Hay Fever), J. Exper. Med. **62**: 733, 1935.
22. Criep, L. H., Schepartz, A. I., and Engel, M.: Relation of pH of Allergenic Extracts to Storage and Skin Reactivity, J. ALLERGY **24**: 158, 1953.
23. Dankner, A., Bukantz, S. C., Johnson, M. C., and Alexander, H. L.: Fractionation of Pollen Extracts by Chromatography. I. Preliminary Studies With Ragweed Extract, J. ALLERGY **22**: 437, 1951.
24. Dent, C. E.: A Study of the Behavior of Some Sixty Amino-Acids and Other Ninhydrin-Reacting Substances on Phenol 'Collidine' Filter-Paper Chromatograms With Notes as to the Occurrence of Some of Them in Biological Fluids, Biochem. J. **43**: 169, 1948.
25. Evans, A. G.: A Buffered Physiologic Salt Solution, J. Infect. Dis. **30**: 95, 1922.
26. Frankel, S., Johnson, M. C., Schiele, A. W., Bukantz, S. C., and Alexander, H. L.: Fractionation of Pollen Extracts. II. Chemical and Paper Chromatographic Investigation of Ragweed Pollen Extracts, J. ALLERGY **26**: 33, 1955.
27. Gay, L. N.: The Effect of Heat and Hydrogen Ion Concentrations on the Extract of Short Ragweed, J. Immunol. **11**: 371, 1926.
28. Grove, E., and Coca, A. F.: Studies in Specific Hypersensitiveness. XV. On the Nature of the Atopens of Pollens, House Dust, Horse Dander and Green Pea, J. Immunol. **10**: 471, 1925.
29. Hampton, S. F., Bukantz, S. C., and Johnson, M. C.: Quantitative Immunologic Studies With Allergens. II. Deterioration of Ragweed Pollen Extracts as Measured by Precipitation, Neutralization and Protein Nitrogen Analysis. The Prevention of Deterioration by Glycerin, J. ALLERGY **20**: 19, 1949.
30. Hampton, S. F., Johnson, M. C., Frankel, S., and Schiele, A. W.: The Preparation of Highly Concentrated Allergenic Extracts by Increasing the Concentration of Extraction. I. Timothy and Low Ragweed Pollens, J. ALLERGY **26**: 16, 1955.
31. Long, W. L., and Teller, I.: Activation of Ultrafiltrates of Ragweed Pollen Extracts by Means of Colloidal Substances, J. ALLERGY **9**: 433, 1938.

32. Ma, T. S., and Zuazaga, G.: Micro-Kjeldahl Determination of Nitrogen. A New Indicator and an Improved Method, Indust. & Engin. Chem. Analyt. Ed. **14:** 280, 1942.
33. Miller, L., and Houghton, J. A.: The Micro-Kjeldahl Determination of the Nitrogen Content of Amino Acids and Proteins, J. Biol. Chem. **159:** 373, 1945.
34. Moore, M. B., Cromwell, H. W., and Moore, E. E.: Studies on Pollen and Pollen Extracts. V. Skin Reactions to Pollen Fractions, J. ALLERGY **2:** 85, 1930.
35. Nelson, N.: A Photometric Adaptation of the Somogyi Method for the Determination of Glucose, J. Biol. Chem. **153:** 375, 1944.
36. Partridge, S. M., and Westall, R. G.: Filter-Paper Partition Chromatography of Sugars. I. General Description and Application to the Qualitative Analysis of Sugars in Apple Juice, Eggwhite, and Foetal Blood of Sheep, Biochem. J. **42:** 238, 1948.
37. Partridge, S. M.: Aniline Hydrogen Phthalate as a Spraying Agent for Chromatography of Sugars, Nature, London **164:** 443, 1949.
38. Portnoy, J., and Sherman, W. B.: Complement Fixation Studies in Ragweed Allergy. I. Quantitative Complement Fixation Studies with Ragweed-Pollen Extract and Homologous Rabbit Antisera, J. ALLERGY **25:** 215, 1954.
39. Rappaport, B. Z.: The Allergic Activity of Proteins Sterilized by Heat, J. ALLERGY **4:** 1, 1932.
40. Rockwell, G. E.: Active Chemical Components of Crude Ragweed Extract, Ohio Med. J. **39:** 128, 1943.
41. Rockwell, G. E.: Empirical Formula, Structural Formula, and Molecular Weight of the Major Antigen in Crude Ragweed Pollen Extract, Ann. Allergy **1:** 43, 1943.
42. Segur, J. B.: "Uses of Glycerin" in Miner, C. S., and Dalton, N. N.: Glycerol, Am. Chem. Soc. Monograph No. 117, 1953.
43. Somogyi, M.: Determination of Blood Sugar, J. Biol. Chem. **160:** 61, 1945.
44. Stier, R. F. E., and Hollister, G. L.: Comparative Study of Pollen Antigens as Determined by Skin Reactions, J. Lab. & Clin. Med. **12:** 1139, 1927.
45. Stull, A., Chobot, R., and Cooke, R. A.: Chemical and Clinical Studies on Pollen (Preliminary Report), J. ALLERGY **1:** 470, 1930.
46. Stull, A., Cooke, R. A., and Chobot, R.: The Allergically Active Substance in Ragweed Pollen. A. Chemical and Biological Study, J. Biol. Chem. **92:** 569, 1931.
47. Stull, A., Cooke, R. A., and Tennant, J.: The Allergen Content of Pollen Extracts. Its Determination and Its Deterioration, J. ALLERGY **4:** 455, 1933.
48. Stull, A., Cooke, R. A., Sherman, W. B., Hebald, S., and Hampton, S. F.: Experimental and Clinical Study of Fresh and Modified Pollen Extracts, J. ALLERGY **11:** 439, 1940.
49. Stull, A., Cooke, R. A., and Glidden, M.: The Protein Nitrogen Unit for Standardization of Inhalant Extracts for Diagnosis and Treatment of Allergic Diseases, J. ALLERGY **8:** 454, 1937.
50. Stull, A., Cooke, R. A., and Chobot, R.: The Allergenically Active Substance in Pollen. A Chemical and Biologic Study of Phleum Pratense (Timothy) Pollen, J. ALLERGY **3:** 341, 1932.
51. Sullivan, C. J., and Vaughan, W. T.: Highly Concentrated Pollen Extracts and Their Deterioration in Various Media, J. ALLERGY **10:** 551, 1939.

CORTISONE OR HYDROCORTISONE IN THE TREATMENT OF SEVERE, INTRACTABLE ASTHMA?

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INTRODUCTION

CORTISONE and hydrocortisone are both readily available for clinical use. The practicing physician naturally wishes to know which hormone is more effective and safer in the quantities in which each agent must be used. Certain experimental data may be of importance. Nelson and associates[1] stimulated the adrenal glands of dogs by giving corticotropin. In the blood flowing in the adrenal veins of these dogs, they found that the predominant hormone was Compound F. Conn and associates[2] found that hydrocortisone (17-hydroxycorticosterone) and corticosterone were the hormones of quantity secreted by the adrenal cortex of dogs. Ingle and Meeks[3] administered cortisone and hydrocortisone by continuous subcutaneous injection to rats. Using weight loss, negative nitrogen balance, glycosuria, atrophy of adrenal cortex and thymus, and gross pathologic changes, such as renal damage and stomach ulcers, as indices of hypercortical activity, they found the quantitative biologic activity of hydrocortisone about twice that of cortisone.

At this meeting last year, Arbesman and Richard[4] and Bickerman and Barach[5] compared the use of cortisone and hydrocortisone in asthma. Arbesman and Richard concluded that the comparable effective dose of hydrocortisone is about two-thirds that of cortisone. Bickerman and Barach showed that hydrocortisone's effect was more rapid and that hydrocortisone produced fewer undesirable effects than cortisone. They stated that hydrocortisone dosage was one-half to two-thirds that of cortisone. In the summer of 1953 it was felt that a controlled study with statistical analysis of dosage figures would be of interest.

METHODS

In April and May, 1951, six patients with intractable asthma severe enough to cause social and economic invalidism were placed on cortisone.

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Repeated attempts to establish an etiological diagnosis had failed in each patient and usual therapy, such as epinephrine, aminophylline, and hospital care, had failed to give adequate relief. From time to time, similar patients were added to the study. The end point in each patient was complete suppression of symptoms. At first enough cortisone was given to abolish symptoms. This is known as the initial clearing dose. The daily dose was then gradually reduced until symptoms began to reappear. A small subsequent increase in daily dosage led to disappearance of symptoms. This dose is called the daily maintenance dose. Repeated efforts to lower this dose have been made. Burrage and associates[6-8] have reported their observations from time to time.

From November, 1953, to June, 1954, eight of the patients who had been maintained symptom-free on daily cortisone were switched to the same daily dose of hydrocortisone. This dose of hydrocortisone was reduced every week by 10 to 20 mg. When asthmatic symptoms began to reappear, the daily dose was then raised slightly until symptoms cleared. This again was called the daily maintenance dose. Repeated attempts were made to lower this effective level. The rest of the patients were continued on cortisone therapy, thereby acting as controls.

From January through March, 1954, seven patients with asthma similar to that of the original group were placed on hydrocortisone. Again the total clearing dose was established, and later the lowest possible daily maintenance dose was found. Again, complete freedom from symptoms was required.

RESULTS

Table I summarizes the data of these experiments. Several points must be considered in analyzing these figures. As has been stated, the clearing dose represents the exact total amount of cortisone or hydrocortisone which was needed for complete subjective and objective clearance of a patient's asthmatic symptoms. The average daily maintenance dose of cortisone or hydrocortisone was obtained by adding together all the daily maintenance doses of either hormone on which the patient was completely free of symptoms and then dividing the total by the number of days. The daily doses on which the patient did have subjective asthmatic symptoms or objective findings were not included. To demonstrate the exactness of this procedure, the following example is cited: One patient came in on a Friday; she was free of symptoms, and her chest was clear on physical examination. She was instructed, therefore, to lower her dose, starting Saturday. She was seen the following Friday. She stated that she had had no asthma, but expiratory wheezes were found throughout her lungs. The daily doses for the previous six days were consequently discarded when her daily maintenance dose was computed.

Casual study of Table I leaves one with the opinion that there is little difference between cortisone and hydrocortisone, milligram for milligram, as far as the clearing dose is concerned but that less hydrocortisone than cortisone is needed in maintenance therapy.

Statistical analysis of the figures in Table I is of interest. When a mean is struck of the initial clearing doses of cortisone of all nineteen patients, it proves to be 1,232 mg. Patient E. Koz. originally, however, was not treated like the rest. She was started on intravenous ACTH and was then placed on 100 mg. of cortisone every twenty-four hours. On this basis, 3,125 mg. of cortisone was required to free her from symptoms. This value of 3,125 is two and one-half times the standard deviation and is, therefore, just on the border line of acceptance as not being an accidental error. In view of this, plus the fact that E. Koz. was not treated like the others, this figure will be discarded.

TABLE I

PATIENT	SEX	AGE	INITIAL CLEARING DOSE IN MG. CORTISONE	INITIAL CLEARING DOSE IN MG. HYDROCORTISONE	AVERAGE DAILY MAINTENANCE DOSE CORTISONE IN MG.	AVERAGE DAILY MAINTENANCE DOSE HYDROCORTISONE IN MG.
M. A.	F	44	850		55	
A. B.	F	60	650		51	
B. B.	F	54	1474		63	
P. B.	M	73	825		65	50
B. C.	F	47	2300		100	82
M. D.	F	57	725		65	44
C. E.	M	55		1150		43
T. G.	M	34	775		76	
E.H.	F	59		1100		54
E. Kos.	F	44	750		84	
E. Koz.	F	48	3125*		67	50
D. M.	M	61	950		73	56
E. M.	F	39	625		63	43
M. M.	F	62	1816		63	
G. Mc.	M	57		1000		50
J. P.	F	43	1375		86	
M. P.	F	62		1950		47
C. R.	M	60	500		117	80
F. S.	F	47	1325		75	
A. W.	F	59	1150		37	31
E. W.	M	64	875		77	
G. W.	F	74		675		55
R. W.	M	49	800		94	
S. W.	F	48		1510		38
N. Z.	F	56	2525		77	

*100 mg. ACTH, intravenous drip.

The mean of the initial clearing doses of cortisone is, then, 1,127 mg. with a probable error of ±137. The mean of the initial clearing doses of hydrocortisone for six patients is 1,231 mg. with a probable error of ±181. Thus, comparison of 1,127 mg. ±137 for cortisone and of 1,231 ±181 for hydrocortisone will be considered. The standard deviation for cortisone is 579, and that of hydrocortisone is 442. Comparing the two means by the Student's distribution test, one finds a probability of 0.7 that they are equivalent. The conclusion that cortisone and hydrocortisone are equally effective as the initial clearing doses appears to be statistically valid.

The mean of the average daily maintenance doses of cortisone in nineteen patients is 73 mg. ±4.2 in contrast to the mean of the average daily maintenance doses of hydrocortisone in fourteen patients, which is 51.6 mg. ±3.8. The

standard deviations are 18 and 14, respectively. Again applying the Student's distribution test, the probability is 0.01 that the difference is random. The difference of the means, therefore, is statistically significant. The conclusion that less hydrocortisone than cortisone is needed in maintenance therapy in severe, intractable asthma is evident.

Taking the eight patients who were maintained first on cortisone and then on hydrocortisone, one finds an average mean difference of 18.9 mg. with a probable error of ±3 in favor of hydrocortisone. The standard deviation is 8.3. Application of the Student's distribution test offers the probability that the difference of the mean being random is only 0.001. The difference is thus statistically valid.

Some might object to these data on the basis that the temporal aspect (duration of therapy) may influence maintenance doses. Some patients, however, have now been on daily cortisone for over four years. In no patient has the daily maintenance dose shifted except temporarily during periods of stress or respiratory tract infections.

COMPLICATIONS

Since maintenance cortisone or hydrocortisone in this type of severe, intractable asthma generally represents more cortisone or hydrocortisone than is normally present in twenty-four hours in the body, effects of hyperadrenocorticism may be anticipated. To date, the most serious complication of hyperadrenocorticism in these patients has been osteoporosis. Osteoporosis has appeared in three patients on maintenance cortisone or hydrocortisone. P. B., a 73-year-old man, has no symptoms, but roentgenograms have showed two compressed fractures of vertebrae after three years of maintenance cortisone and eight months of maintenance hydrocortisone. B. B., a 54-year-old woman, developed symptomatic osteoporosis with compressed vertebral fractures after two months of maintenance hydrocortisone therapy. W. G., a 60-year-old man, developed compressed vertebral fractures after eight months of maintenance cortisone therapy.

Other serious complications encountered were all in W. G., whose duodenal ulcer bled on Jan. 14, 1954. This was controlled by subtotal gastrectomy. He did well during the immediate postoperative period. Unfortunately, while at home in March, 1954, he developed a masked pneumonia which led to his death within forty-eight hours.

SUMMARY AND CONCLUSIONS

1. There appears to be little to choose between cortisone and hydrocortisone for the purpose of clearing symptoms of severe, intractable asthma by the methods described.

2. In maintenance treatment of intractable asthma, hydrocortisone is more effective, milligram for milligram, than cortisone.

3. Maintenance dosage of either cortisone or hydrocortisone in asthma may cause the more serious effects of hyperadrenocorticism, including osteoporosis.

REFERENCES

1. Nelson, D. H., Reich, H., and Samuels, L. T.: Isolation of a Steroid Hormone From the Adrenal Vein, Science **111**: 578, 1950.
2 Conn, J. W., Lawrence, H. L., and Fajans, S. S.: The Probability That Compound F (17-Hydroxycorticosterone) is the Hormone Produced by the Normal Adrenal Cortex, Science **113**: 713, 1954.
3. Ingle, D. J., and Meeks, R. C.: Comparison of Some Metabolic and Morphologic Effects of Cortisone and Hydrocortisone Given by Continuous Injection to Rats, Am. J. Physiol **170**: 77, 1952.
4. Arbesman, C. E., and Richard, N. B.: Prolonged Cortisone and Hydrocortisone Therapy, J. ALLERGY **25**: 306, 1954.
5. Bickerman, H. A., and Barach, A. L.: Comparative Results of the Use of ACTH, Cortisone, and Hydrocortisone in the Treatment of Intractable Bronchial Asthma and Pulmonary Emphysema, J. ALLERGY **25**: 312, 1954.
6. Burrage, W. S., Irwin, J. W., and Gibson, J. S.: Maintenance Cortisone in Severe Bronchial Asthma, J. ALLERGY **23**: 310, 1952.
7. Burrage, W. S., and Irwin, J. W.: The Role of Cortisone in the Treatment of Severe Bronchial Asthma, New England J. Med. **248**: 679, 1953.
8. Irwin, J. W., Henneman, P. H., Wang, D. M. K., and Burrage, W. S.: Maintenance Cortisone in Intractable Asthma, J. ALLERGY **25**: 201, 1954.

THE PATHOLOGY OF BRONCHIAL ASTHMA. I. THE SIGNIFICANCE OF MEMBRANE CHANGES IN ASTHMATIC AND NON-ALLERGIC PULMONARY DISEASE

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IN THE establishment of an anatomic diagnosis of bronchial asthma, considerable emphasis is placed on the plurality of histopathologic lesions. Several authors[1-3] have stressed this requisite and have defined the various alterations of the pulmonary tree which comprise the pathologic pattern. The pathologic changes include hyperplasia of mucous glands, hyperemia and edema of the mucosa, eosinophilic infiltration of the mucosa, hypertrophy of bronchiolar musculature, and formation of mucous plugs. The actual thickness of bronchiolar walls may be considerably increased by the summation of alterations. All such phenomena are essentially reversible and many are transient, so that each alone is of limited significance. Some authors have attached great importance to "invariable thickening and hyalinization" of the bronchiolar basement membrane.[4] Because of its relatively permanent character, it alone might be considered as a "pathognomonic" feature of pre-existing bronchial asthma.

The present study was undertaken to elucidate the value of this change in the basement membrane as a feature of bronchial asthma. Its precise value as a histopathologic criterion of bronchial asthma requires that two standards be satisfied. First, the change must be present in all cases of established bronchial asthma and, second, it must not occur in other nonallergic, nonasthmatic, pulmonary conditions. Failure to meet these standards would preclude it from use by itself as a diagnostic criterion of bronchial asthma. With this in mind, the incidence and severity of thickened basement membranes were assessed in lungs from a variety of patients in order to determine their degree of specificity and type of distribution.

MATERIALS AND METHODS

The tissues studied in this investigation were obtained from 116 autopsies performed at the University Hospitals during the period 1949-1950, and from 107 lungs removed surgically because of variety of disease processes, mainly carcinoma, tuberculosis, and bronchiectasis. The cases were carefully evaluated clinically and were accepted as a group when the absence of either asthma or a personal history of any allergic state in the present or past had been established. A few cases were deleted because pertinent clinical data were inadequate. Sections from the lungs of twenty-two cases of established bronchial asthma, obtained from the files of the Department of Pathology, were also examined; the clinical diagnosis in all these was unequivocal.

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TABLE I. THE INCIDENCE OF THICKENED PULMONARY BASEMENT MEMBRANES IN PATIENTS WITHOUT CLINICAL EVIDENCE OF ALLERGY: A SUMMARY OF 116 POSTMORTEM AND 107 SURGICAL SPECIMENS

AGE (YEARS)	116 POSTMORTEM SPECIMENS				107 SURGICAL SPECIMENS			
	NO. OF CASES	MEMBRANES NORMAL	MEMBRANES THICKENED	PER CENT THICKENED	NO. OF CASES	MEMBRANES NORMAL	MEMBRANES THICKENED	PER CENT THICKENED
0 to 30	32	29	3	9	33	26	7	21
31 to 60	39	28	11	28	65	40	25	38
61 to 90	45	32	13	29	9	6	3	33
Total	116	89	27	23	107	72	35	32

In preparation of the specimens, the pieces of tissues of autopsied cases were all fixed in Formalin diluted to 1 in 10 without neutralization. The surgically removed lungs were fixed en masse by pouring the same strength solution of Formalin into the main bronchus of the insufflated organ until all the air had been replaced with fluid. When satisfactorily fixed, the tissues were embedded in paraffin, cut at a thickness of 8 micra, and regularly stained with hematoxylin and eosin. In selected specimens, the slides of the Formalin-fixed tissues were postchromated by the technique of Mullen and McCarter[5] and stained with both Mallory's phosphotungstic hematoxylin stain[6] and Masson's trichrome stain[7] to accentuate the histologic features of the muscular tissue and submucosa. The MacManus[8] modification of the periodic acid-leukofuschin stain was used to define several histologic structures, especially the limits of the basement membrane.

RESULTS

It was observed that, in the lungs from the routine autopsies, 23 per cent had thickened membranes, whereas among surgically removed lungs an incidence of 32 per cent was found (Table I), and among the asthmatic patients the occurrence was 100 per cent. Although the total proportion of lungs with thickened membranes was not very different between the autopsy and surgical groups, the age distribution was very significantly dissimilar. Thickened membranes were rarely found in autopsied lungs in the first three decades, with a considerable increase for the later decades in this group. In surgical specimens, on the contrary, the percentage with thickened membranes was high during the early decades and much higher in later life. No significant variation in relation to sex was detected. It was not feasible to grade the degree of thickening, as it was often patchy and irregular, even in the same transverse section of a small bronchus or bronchiole. A similar lack of uniformity was also observed in many cases of asthma.

In the lungs removed surgically, each specimen was the site of some variety of chronic inflammatory, neoplastic, or cystic condition which had necessitated elective surgery. The sections evaluated were taken at a distance from the involved area. Detailed analysis of lungs of this series with and without altered membranes revealed no peculiar distribution of the type of disease encountered in these arbitrary groups. It was considered that, if a basic variety of disease were especially to cause thickened membranes, such disease would preponderate among the lungs with thick membranes. The distribution in the present series affords no conclusive evidence (Table II), because in the cases with tuberculosis and bronchiectasis chronic inflammation was characteristic, and in many cases with neoplasia there was associated chronic inflammation.

On the other hand, in the autopsy lungs with thickened membranes, a large proportion showed concomitant chronic pathologic changes, such as bronchiectasis, bronchitis, tuberculosis, and carcinoma. This type of lesion (Table III) was much less frequent in the lungs that had normal basement membranes. There was an increase, however, in the incidence of chronic pulmonary disease with advancing age; the percentage at the upper extreme of life was 61 per

TABLE II. THE TYPE OF CHRONIC LESIONS IN 107 SURGICALLY REMOVED LUNGS IN RELATION TO BASEMENT MEMBRANE ALTERATION

DISEASE	CASES	MEMBRANE THICKENED	MEMBRANE NORMAL
Tuberculosis	55	17	38
Bronchiectasis	27	9	18
Carcinoma	15	5	10
Chronic inflammatory*	10	4	6
Total	107	35	72

*These include two cases each of chronic abscess, coccidioidomycosis, and virus pneumonitis, and one each of lipid pneumonia, simple cyst, and unexplained chronic fibrosis.

TABLE III. THE RELATION OF PULMONARY LESIONS, AGE, AND THICKENED BASEMENT MEMBRANES IN 116 AUTOPSIED LUNGS

	NORMAL BASEMENT MEMBRANE				THICKENED BASEMENT MEMBRANE			
AGE GROUP	CASES	NO PULMONARY DISEASE	PULMONARY DISEASE	PER CENT* WITH PULMONARY DISEASE	CASES	NO PULMONARY DISEASE	PULMONARY DISEASE	PER CENT* WITH PULMONARY DISEASE
0 to 30	29	29	0	0	3	2	1	33
31 to 60	28	23	5	20	11	6	5	45
61 to 90	32	21	11	34	13	5	8	61
Total	89	73	16	23	27	13	14	52

Pulmonary lesions included all chronic processes, such as bronchiectasis, chronic pneumonitis, lung abscess, and tuberculosis. Acute pathologic processes, such as bronchopneumonia and pulmonary edema, are excluded.

*Percentage figures are rounded out.

cent, and at the lower age limits fewer patients had altered membranes. It was apparent that both the process of aging and chronic pulmonary disease contributed to the thickening of the membranes. As previously indicated, the occurrence of chronic lesions was most conspicuously seen in the surgical specimens.

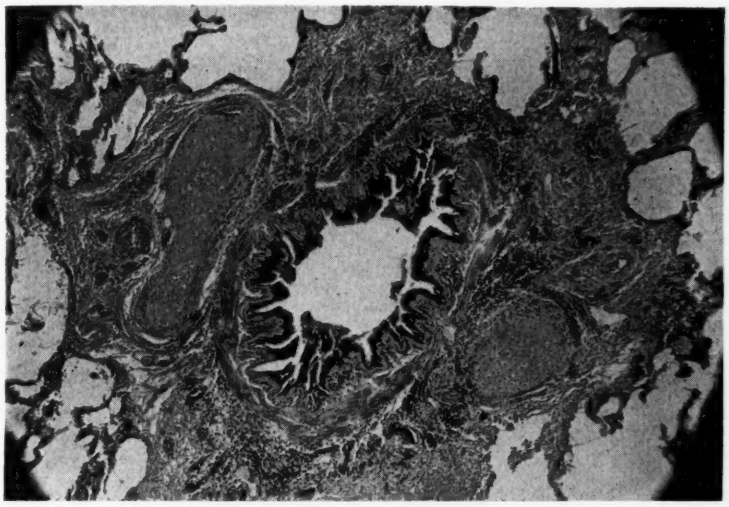

Fig. 1.—Fibrocaseous tuberculosis. No personal history of allergy. Note thickness of basement membrane.

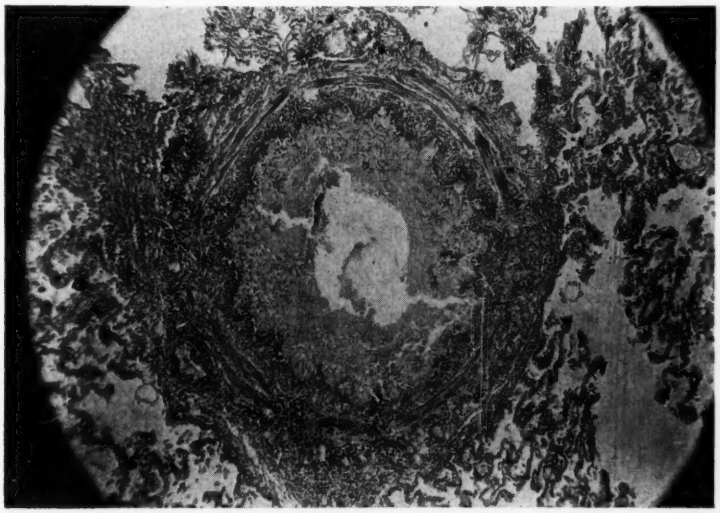

Fig. 2.—Bronchial asthma. Note mucus, diffuse inflammation of wall, and thick basement membrane.

Not only was the thickening in the basement membrane common in nonallergic chronic disease of the lungs, but the extent of it was often as great as in cases of all well-established, long-standing bronchial asthma. In Fig. 1

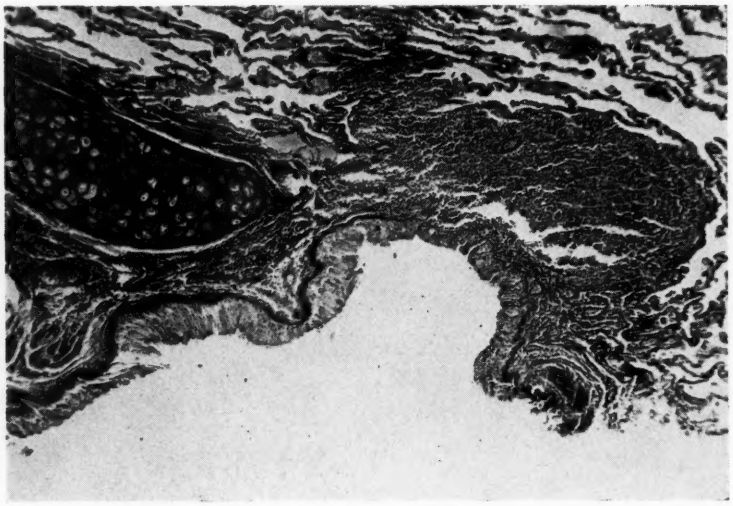

Fig. 3.—Bronchiectasis. Note patchy variation in thickness of basement membrane.

Fig. 4.—Nonasthmatic. Note that thickness has extended to atrial area.

is illustrated a uniformly thickened, wavy basement membrane in the bronchiole of a lung removed because of extensive fibrocaseous tuberculosis without coexisting allergy. This lesion is as extensive as that seen in bronchial asthma (Fig. 2). On the other hand, the lesion may be patchy in distribution, with considerable variation in the extent of thickening (Fig. 3). A similar irregularity in the distribution of the lesion is frequently observed in cases of asthma. The thickening was not confined to the larger bronchioles, but extended down into both the atria and infundibular (Fig. 4). Other alterations considered peculiar

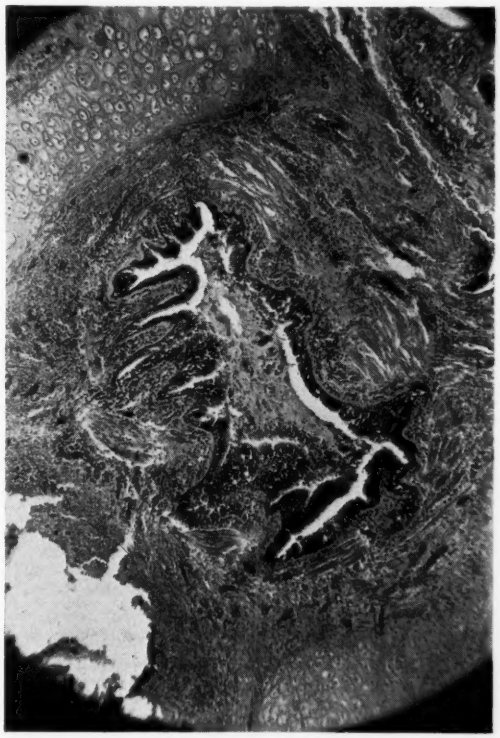

Fig. 5.—Nonasthmatic. Lobe removed for tuberculosis. Note mucus, inflammation of wall, and thick basement membrane.

to asthma were also encountered; there were accumulation of mucus, hyperplasia of mucous glands, and squamous metaplasia accompanying the several varieties of nonallergic pulmonary disease (Fig. 5). Yet, in all the tissues examined from the nonallergic groups of cases, the absence of eosinophilic infiltration was evident. Gay[2] has emphasized the occurrence of interstitial eosinophilia in the bronchiolar tissue of his cases.

DISCUSSION

It is evident that the occurrence of thickened basement membranes is not confined to these cases of bronchial asthma. The incidence of the membrane alteration is related to many chronic pulmonary diseases, especially in later

ages, so that the pathogenesis is not specifically dependent upon allergic phenomena and may be a manifestation of the interstitial reactions which characterize serous inflammations as described by Altshuler and Angevine.[9] The development of identical thick membranes in the mucosa of nasal polyps and of sinuses affected by chronic sinusitis also suggests this casual relationship in conditions where large quantities of acid mucopolysaccharide ground substances are elaborated.

The ubiquity of this change precludes its use as a characteristic of bronchial asthma. However, the circumstances of its development in bronchial asthma, in the absence of other chronic pulmonary disease, are peculiar to asthma. The predisposition in uncomplicated bronchial asthma toward development of thickened membranes is emphasized by Thomson's[10] report of "asthmatic" changes in the respiratory type epithelium found in a dermoid cyst of a woman who died of bronchial asthma. The degree of specificity of this reaction in asthma is illustrated by this case and by several of those discussed by Winer and his associates.[11] Nevertheless, the specificity of the thickened membrane as a diagnostic criterion is equivocated by the frequency with which the same change occurs in other chronic inflammatory diseases of the lung. The asthmatic state is clinically manifest because of diffuse respiratory dysfunction due to the changes which are characteristic of the allergic inflammation causing obstruction of the airway. The inflammatory phenomena are transient, but produce a residual thickened basement membrane which, however, may also be the consequence of other chronic inflammatory diseases.

SUMMARY

There is a frequent occurrence of thickened basement membranes in nonallergic, chronically diseased lungs. The thickened basement membrane is not a peculiarity of bronchial asthma alone.

REFERENCES

1. Huber, H. L., and Koessler, K.K.: Pathology of Bronchial Asthma, A. M. A. Arch. Int. Med. 30: 689, 1922.
2. Gay, L. N.: The Diagnosis and Treatment of Bronchial Asthma, Baltimore, 1946, Williams & Wilkins Company, p. 44.
3. Craige, B., Jr.: Fatal Bronchial Asthma, A. M. A. Arch. Int. Med. 67: 399, 1941.
4. MacDonald, I. G.: Local and Constitutional Pathology of Bronchial Asthma, Ann. Int. Med. 6: 253, 1932.
5. Mullen, J. P., and McCarter, J. C.: Mordant Preparing Formaldehyde-fixed Neuraxis Tissue for Phosphotungstic Acid Hematoxylin Staining, Am. J. Path. 17: 289, 1941.
6. Mallory, F. B.: A Contribution to Staining Methods, J. Exper. Med. 5: 15, 1900-1901.
7. Masson, P.: Carcinoids (Argentaffin-cell Tumors) and Nerve Hyperplasia of Appendicular Mucosa, Am. J. Path. 4: 181, 1928.
8. MacManus, J. F. A.: Periodic Acid Routine Applied to Kidney, Am. J. Path. 24: 643, 1948.
9. Altshuler, C. A., and Angevine, D. M.: Acid Mucopolysaccharide in Degenerative Disease of Connective Tissue With Special Reference to Serous Inflammation, Am. J. Path. 27: 141, 1951.
10. Thomson, J. G.: Bronchial Asthma Showing the Asthmatic Reaction in an Ovarian Teratoma, J. Path. & Bact. 57: 213, 1945.
11. Winer, B. M., Beokey, J. F., and Segal, M. S.: A Clinicopathologic Study of Bronchial Asthma With Consideration of Its Relationship to the General Adaptation Syndrome, Ann. Int. Med. 33: 134, 1950.

ATMOSPHERIC POLLEN COUNTS IN TORONTO, CANADA

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FROM the period, Dec. 7, 1953, to Dec. 6, 1954, daily atmospheric pollen counts were done at The Hospital for Sick Children, Toronto. No such study has been done in this area since the one conducted by LaRush[1] more than twenty years ago.

TECHNIQUE

The counts were done by means of the standard sampling device manufactured by the Wilkens-Anderson Company of Chicago,[2] using standard microscope slides covered with a thin coating of soft petrolatum jelly (75 per cent U.S.P. petrolatum, 25 per cent U.S.P. mineral oil). This apparatus was placed on the roof of the hospital, which is eleven stories high, in a relatively unobstructed position, although a superstructure about thirty feet away caused slight obstruction from a northerly direction. There are no surrounding tall buildings which cause any obstruction. The slide was replaced daily at 11:15 A.M. and examined under a binocular microscope, without stain. The area of the cover slip, 4.4 sq. cm., was counted, but results were reduced to the number of pollen granules per square centimeter for reporting.

RESULTS

The results of the counts are shown in Figs. 1, 2, and 3. From Fig. 1, it is seen that the tree season extends from the first week of April to the second week of July, the grass season from the fourth week of May to the end of July (although some grass pollen was found until the middle of September), and the weed season from the beginning of August to the end of September.

Fig. 2 demonstrates that the important tree pollens, numerically, are birch, elm, maple, oak, pine, and poplar. Elm and poplar pollens are predominant through April; birch through May; maple through April and May; oak through April, May, and the first half of June; and pine largely from the middle of May to the middle of July. Pine pollens gave the highest counts of all the tree pollens and accounted for practically all the tree pollen seen after the first of June.

Fig. 3 reveals that ragweed is the only weed pollen present to an appreciable extent. Very few granules of dandelion pollen (included in the compositae) were seen.

Correlation of Counts With Skin Tests to Pollens in the Toronto Area.—Pollens are clinically very important in the Toronto area, where hay fever and asthma, during the pollen seasons, are very common. Skin reactions to extracts

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of tree pollens are very common, as would be expected from the high pollen counts, and are commonly obtained with alder, ash, and willow, in spite of their low counts obtained in this study. The very high counts obtained with pine pollen came as a distinct surprise. Since pine pollen is not generally considered to be allergenically active, we have never skin tested with this pollen and, therefore, cannot make any comment on skin testing with this agent.

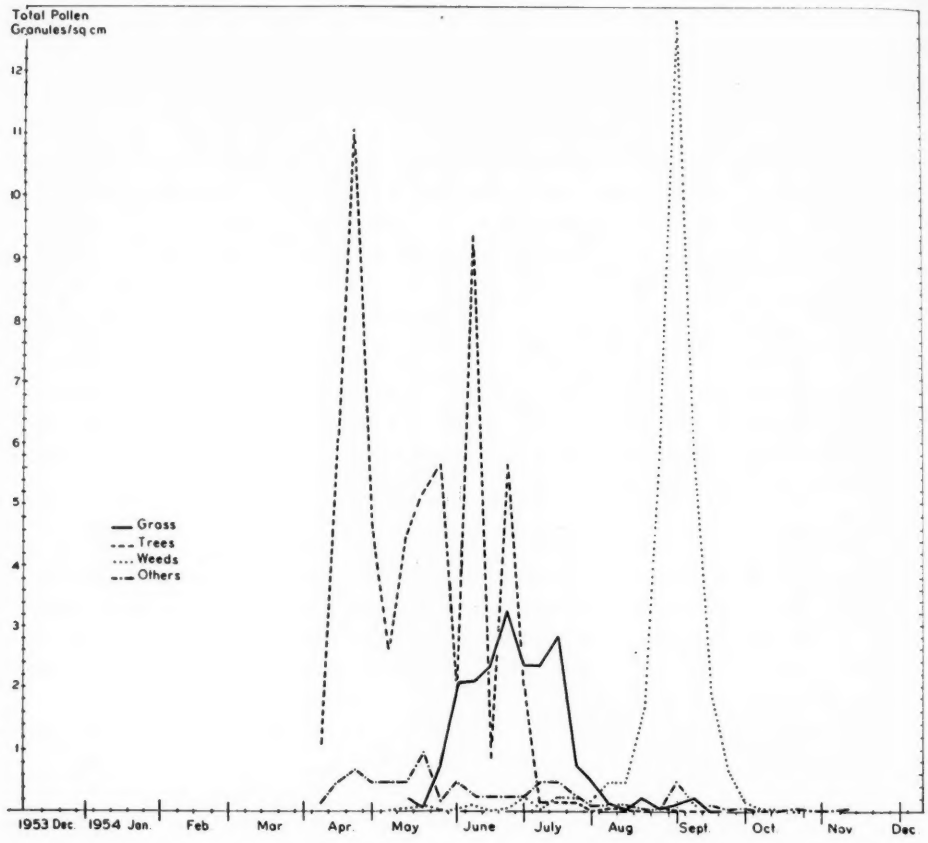

Fig. 1.—Total tree, grass, and weed pollen counts, expressed as number of pollen granules per square centimeter of exposed slide. The average daily count for each week is charted for purposes of simplification.

From Fig. 3, it is obvious that ragweed accounts for the major portion of weed pollens in this area. In spite of this, positive skin tests are extremely common with cocklebur, dandelion, goldenrod, goosefoot, mugwort, pigweed, plantain, and Russian thistle, and one of us (C. C. W.) feels very definitely that patients reacting to these weeds have better clinical results if treated with them than if treated with ragweed alone.

Correlation Between Pollen Counts and Observations on the Weather.—Certain observations on the weather are of interest. It was noted that if the mean daily temperature fell 10 degrees or more, the daily pollen count almost

always showed a precipitous fall. Similarly, heavy precipitation caused definite falls in the daily pollen counts, although light precipitation made no apparent difference. No correlation could be found between the daily pollen counts on the one hand and average wind speed or prevailing wind direction on the other. While it is usually stated that ragweed pollen disappears from the

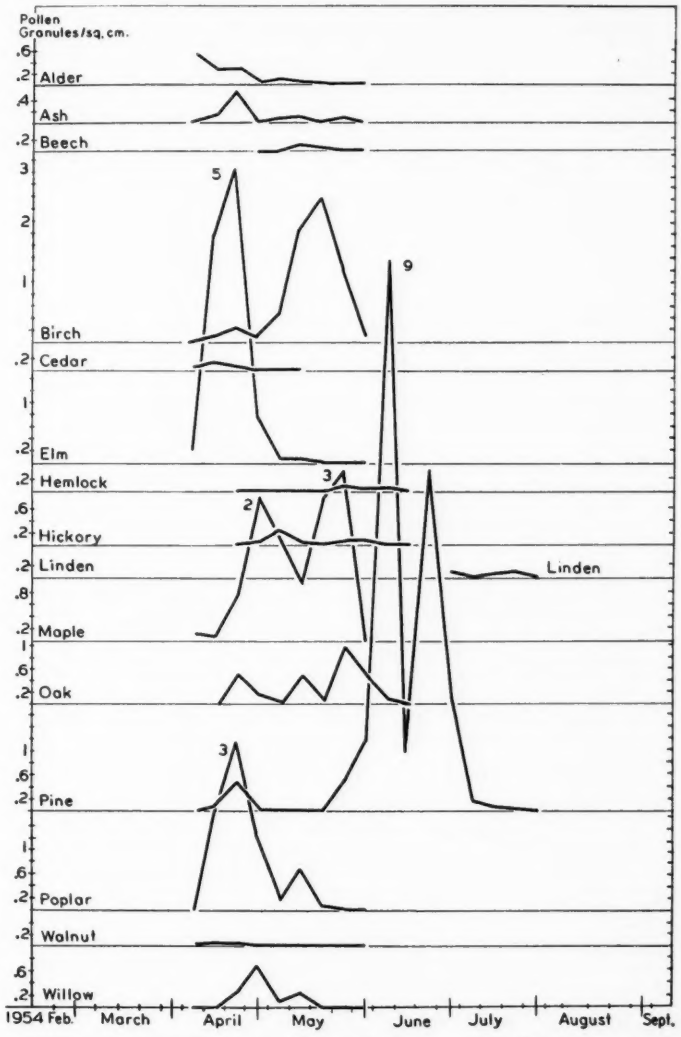

Fig. 2.—Pollen counts of all trees whose pollens were identified, expressed as number of pollen granules per square centimeter of exposed slide. The average daily count for each week is charted for purposes of simplification.

air at the time of the first frost, this year the ragweed count fell to insignificant levels by September 25 and the first frost was not until October 7.

Distribution of Trees, Grasses, and Weeds in Southern Ontario.—The incidence of the various types of pollens obtained by pollen counts depends on many factors. Obviously, one of the most important factors is the prevalence

of the pollen-producing plants in the area in which the counts are being done. Therefore, a knowledge of the distribution of these plants is important in order to generalize on the incidence of pollen granules in neighboring areas.

Trees: Southern Ontario (Fig. 4) has a wide variety of tree species. For the purposes of description, the map is divided into four areas, formed, in part, by the geological structure of the land. The southern boundaries of Area 4 are those of the granite Canadian Shield coming down to Lake Ontario around Kingston. Area 1 is approximately the northern limit of the so-called Carolinian zone of vegetation, extending up from the south into Ontario.

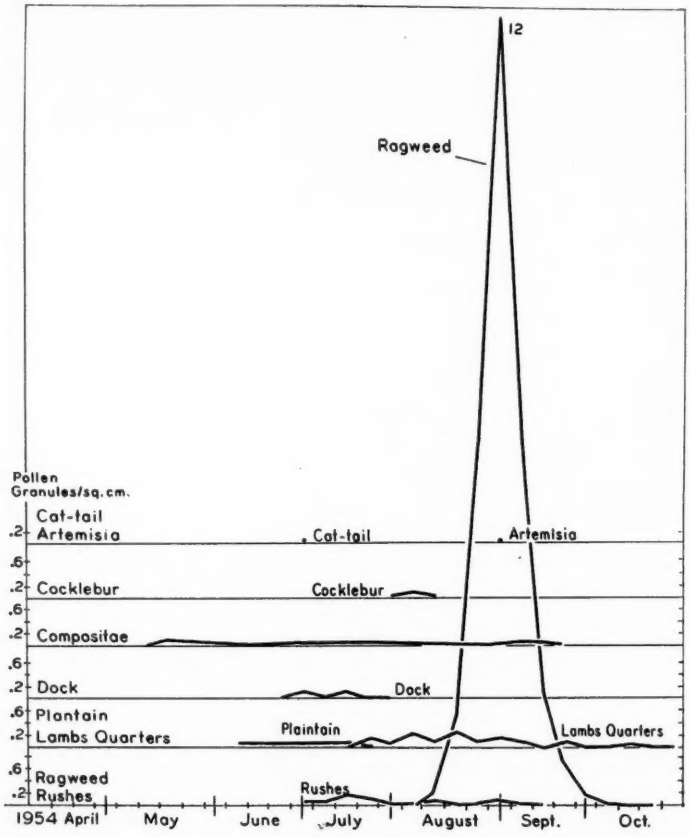

Fig. 3.—Pollen counts of all weeds whose pollens were identified, expressed as number of pollen granules per square centimeter of exposed slide. The average daily count for each week is charted for purposes of simplification.

The distribution of the different genera whose pollen was obtained on the slides will be discussed briefly.

The commonest alder *(Alnus rugosa)* occurs over the entire area, mainly in swamps and by streams. No other alders are present in quantity.

The white ash *(Fraxinus americana)* and the black ash *(F. nigra)* are the most common species of this genus. They occur over the entire map area, but are more prevalent in the southern parts.

The beech *(Fagus grandifolia)*, while covering the whole map area, is most common in Areas 1 and 2.

The white birch *(Betula papyrifera)* and the yellow birch *(B. lutea)* extend over the entire map area. The former is found in quantity in the northern section (Area 4).

The eastern white cedar *(Thuja occidentalis)* is the most common cedar. It is found mainly in swampy or moist areas.

White elm *(Ulmus americana)* is the most common species of its genus in southern Ontario. It extends over the entire area, but is more plentiful in Area 2. Two less common species, slippery elm *(U. rubra)* and rock elm *(U. Thomasi)* are also found.

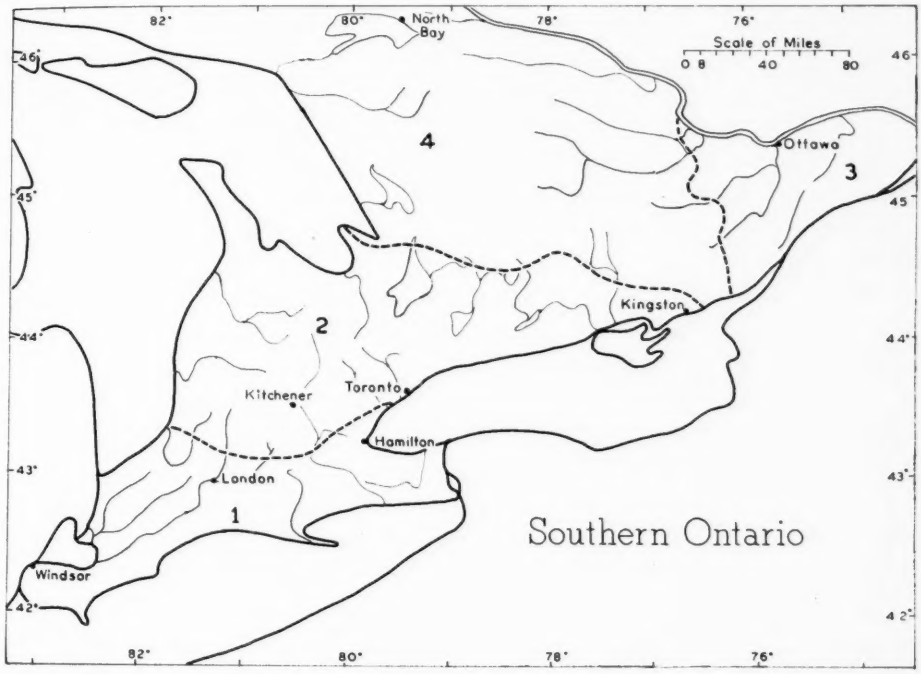

Fig. 4.—Map of southern Ontario, designating the four areas of vegetation referred to in the text.

The eastern hemlock *(Tsuga canadensis)* is found over the whole area.

The hickories are confined to the extreme southern parts of the province (Areas 1 and 2). The bitternut hickory *(Carya cordiformis)* and the shagbark hickory *(C. ovata)* are the most common. The former extends through Areas 1 and 2; the latter is confined to Area 1.

The only native linden or basswood *(Tilia americana)* is found chiefly in Areas 1 and 2, but is distributed over the whole map area.

Maples undoubtedly constitute a larger proportion of the trees over the whole southern Ontario area. The sugar maple *(Acer saccharum)*, silver maple *(A. saccharinum)*, and red maple *(A. rubrum)* are the most common. In the cities, the Norway maple *(A. platanoides)* is planted most extensively.

Among the oaks, there are several species found in southern Ontario, but the following are most frequent: white oak *(Quercus alba)*, northern red oak *(Q. borealis)*, and bur oak *(Q. macrocarpa)*. While these cover the entire map area, they are much more common in the southern section (Area 1).

The two most common species of pine are the white pine *(Pinus strobus)* and the red pine *(P. resinosa)*. Both are found in largest quantity in Area 4, but extend over the whole map area.

The most common poplars in southern Ontario are the aspen *(Populus tremuloides)*, large-toothed aspen *(P. grandidentata)*, and the balsam poplar *(P. balsamifera)*. These species are spread over virtually the whole map area, but are much more common in the northern section, particularly Area 4.

The black walnut *(Juglans nigra)*, the most common walnut, is not plentiful and is confined almost to Area 1. The butternut *(J. cinerea)* is not plentiful either. It is mostly in Area 1, but extends through Area 2.

Many willow species are found. They cover the entire map area, usually occurring, however, in scattered areas along river valleys and streams. Some varieties are also planted for ornamental use.

In this area, there are no trees in quantity from which pollen was not obtained in this study.

In summary, Area 1 is composed of a wide variety of broad-leaved trees, with beech, sugar and red maples, lindens, and oaks predominating. Lesser amounts of hickories, walnuts, ash, and other species occur.

Area 2 is quite similar. Sugar maples and beeches are still dominant. With them are lindens, white elms, yellow birches, and some ash, red maple, and oak trees. Some white and red pines also occur.

Area 3 is very similar to Area 2, but there is a larger proportion of pine.

Area 4 has sugar maples and pines in quantity. Here the poplars and white birches become more frequent, the oak and beech trees much rarer.

Grasses: It is impossible to reach any general conclusion about the grasses in southern Ontario, since so many species are spread over the entire area. However, they are more common in the southern parts, which are more agricultural, than in the rocky, wooded areas of the north. The most common are timothy *(Phleum pratense)*, Kentucky bluegrass *(Poa pratensis)*, and orchard grass *(dactylis glomerata)*.

Weeds: The generalization about grasses applies also to the weeds. The commonest weeds are the ragweeds—short ragweed (Ambrosia elatior), and tall ragweed *(A. trifida)*, the former being much more common.

The plantains *(Plantago major, P. rugelii Dcne,* and *P. lanceolata)* and lamb's-quarters *(Chenopodium album* and *C. glaucum)* are also very common. Occurring to a lesser extent, but still frequent, are the artemisias *(Artemisia biennis* and *A. vulgaris)*, the docks *(Rumex acetosella, R. crispus,* and *R. obtusifolius)*, amaranthus *(Amaranthus retroflexus)*, cocklebur *(Xanthium pennsylvanicum)*, rushes *(Juncaceae)*, and cattails *(Typha)*.

SUMMARY

Daily pollen counts for the year 1954 in Toronto, Canada, are reported. The tree season is predominantly from the first of April into the second week of July, but after June 1 practically the only tree pollen seen is that of pine. The grass season is predominantly from the fourth week of May through July, but grass pollen is found until the middle of September. The weed season is through August and September, with the peak at the beginning of September.

The authors express their thanks to Professor J. H. Soper and Mrs. M. L. Heimburger, Department of Botany, University of Toronto, and to Professor F. H. Montgomery, Department of Botany, Ontario Agricultural College, Guelph, for their assistance in assembling the botanical data.

REFERENCES

1. LaRush, F.: Pollen Content of the Air in Toronto, Canada, 1932, J. ALLERGY **5**: 306, 1934.
2. Durham, O. C.: The Volumetric Incidence of Atmospheric Allergens. IV. A Proposed Standard Method of Gravity Sampling, Counting and Volumetric Interpolation of Results, J. ALLERGY **17**: 79, 1946.

ATMOSPHERIC MOLD COUNTS IN TORONTO, CANADA

C. COLLINS-WILLIAMS, M.D.,* AND C. A. BEST, M.A.,** TORONTO, ONTARIO

FROM the period, Dec. 7, 1953, to Dec. 6, 1954, daily atmospheric mold counts were done at The Hospital for Sick Children, Toronto. No such study has ever been reported from this area.

TECHNIQUE

The counts were done by means of the standard sampling device manufactured by the Wilkens-Anderson Company of Chicago,[1] using standard microscope slides covered with a thin coating of soft petrolatum jelly (75 per cent U.S.P. petrolatum, 25 per cent U.S.P. mineral oil). This apparatus was placed on the roof of the hospital, which is eleven stories high, in a relatively unobstructed position, although a superstructure about thirty feet away caused slight obstruction from a northerly direction. There are no surrounding tall buildings which cause any obstruction. The slide was replaced daily at 11:15 A.M., and examined under a binocular microscope, without stain. The area of the cover slip, 4.4 sq. cm., was counted, but results were reduced to the number of mold granules per square centimeter for reporting.

In addition, a standard sized Petri dish containing modified Mehrlick's medium, as recommended by the American Academy of Allergy, was exposed for twelve minutes each morning at about 11:00 A.M. on an unobstructed window sill at the third-floor level of the hospital, the window having a western exposure on the east side of a wide thoroughfare running north and south. This culture was maintained at room temperature and examined about one week later. The molds were identified by macroscopic examination and by subculture and/or microscopic examination when necessary.

RESULTS

The results of the counts are shown in Figs. 1 and 2. The most important molds, numerically, are alternaria, hormodendrum, and yeasts, although smuts were quite prominent during July and August. The peak season for molds was from the middle of July to the middle of October. Practically none were found from the beginning of January to the middle of June.

Correlation of Counts With Skin Tests to Molds in the Toronto Area.—Although it is difficult to correlate clinical symptoms with atmospheric mold counts, both because most patients who show positive skin tests to molds react to

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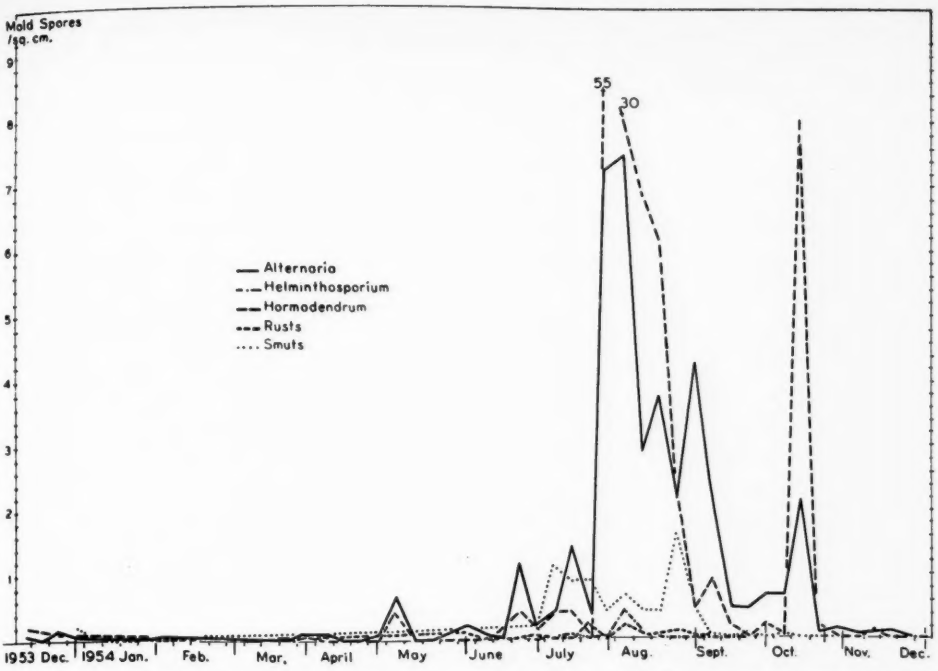

Fig. 1.—Total mold spore counts expressed as number of mold spores per square centimeter of exposed slide. The average daily count for each week is charted for purposes of simplification.

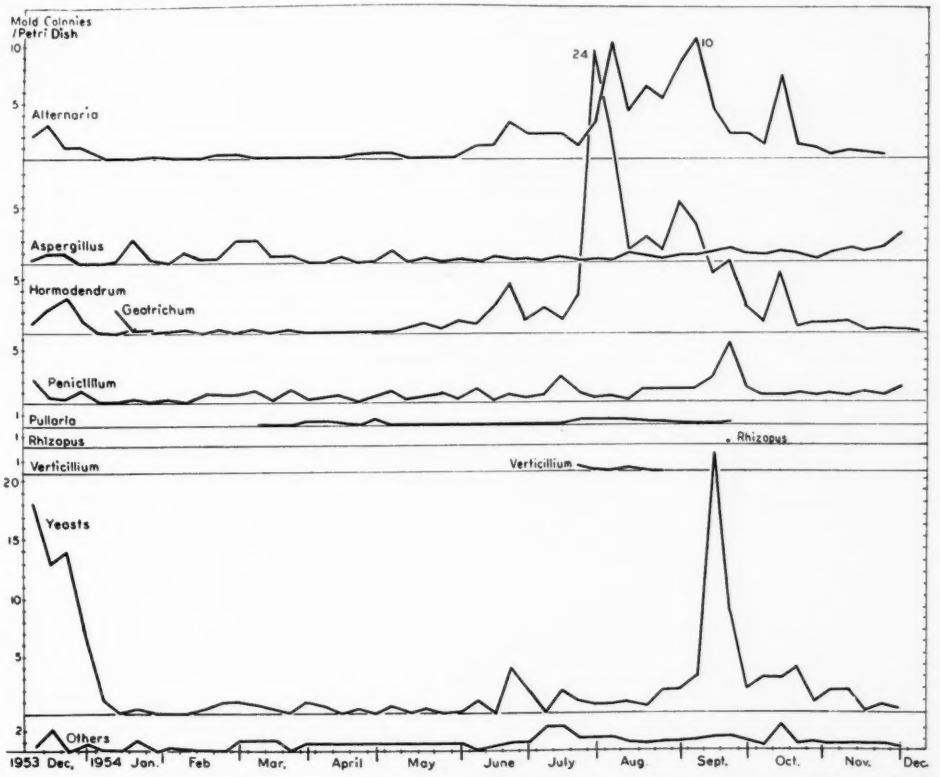

Fig. 2.—Total mold colonies per Petri dish. The average daily count for each week is charted for purposes of simplification.

many other substances also and because the peak mold counts occur during the pollen season, positive skin reactions to molds are very common in this area, especially to alternaria, hormodendrum, and yeasts, but also to *Aspergillus fumigatus, Penicillium notatum,* and *Monilia albicans.* Since we have had no experience with skin tests to the other molds found in this study or with rusts or smuts, no comment can be made on these allergens.

Correlation Between Mold Counts and Observations on the Weather.—An attempt was made to correlate the slide and plate mold counts with changes in the weather. Unlike the changes observed with pollens, there was no correlation between mold counts and a 10-degree drop of mean daily temperature, the mold counts rising on the colder days as frequently as they fell. Similarly, no correlation was found between the mold counts, on the one hand, and precipitation, average wind speed, or prevailing wind direction on the other, with one exception. This one exception occurred when hurricane Hazel struck Toronto. On this day, the mold counts fell to zero and remained negligible for a whole week. During this week, there was practically no additional precipitation and the average daily temperature was approximately 15 degrees lower than it had been prior to the hurricane.

SUMMARY

Daily mold counts, using both exposed slides and Petri dishes, from Dec. 7, 1953, to Dec. 6, 1954, in Toronto, Canada, are reported. The most important molds, numerically, are alternaria, hormodendrum, and yeasts, the peak season being from the middle of July to the middle of October. Smuts are quite prominent during July and August.

REFERENCE

1. Durham, O. C.: The Volumetric Incidence of Atmospheric Allergens. IV. A Proposed Standard Method of Gravity Sampling, Counting and Volumetric Interpolation of Results, J. ALLERGY 17: 79, 1946.

A NEW MICRO SEITZ TYPE FILTER

MARGARET B. STRAUSS, M.S., AND W. C. SPAIN, M.D., NEW YORK, N. Y.

IT IS often necessary to sterilize small quantities of serum or therapeutic solutions without appreciable loss of volume. With the available filters, this is almost impossible. The difficulty with the micro Seitz type filter[1] is that it utilizes negative pressure. When too great a vacuum is applied, frothing is produced, since the solution is blown out before a drop has had a chance to filter through. If, on the other hand, the negative pressure is controlled to prevent frothing, then the drops which filter through are often dried up before the next drop forms; this is especially true in the case of serum filtration. The Swinny[2] adapter overcame the difficulties inherent in the micro Seitz filter utilizing negative pressure. The Swinney adapter is actually a micro Seitz type filter utilizing pressure from the hand onto the syringe plunger to force the liquid through the Seitz pad. While this has been an improvement over the negative-pressure micro Seitz filter, the Swinney adapter has the disadvantage that appreciable quantities of serum or liquid are often lost through seepage between the piston and barrel of the Lok syringe by the amount of pressure often necessary to force serum through the Seitz pad. There is also the ever-present danger of breakage of the glass plunger due to excessive pressure. Also, the time and physical effort involved in forcing the serum through are often appreciable.

In order to overcome the aforementioned difficulties in filtering small quantities of serum or other biologic solutions, the micro Seitz type filter for positive pressure was made at our request by Mr. S. A. Axelrod of Allergists Supply Co.,* as shown in Figs. 1 and 2.

This new filter consists essentially of a lower section, to one outer end of which is attached a hypodermic needle. The inside of the section has a funnel-like appearance, on which is placed a washer, and a wire screen which supports the 10 mm. Seitz filter pad. The center section then screws down into this lower section until it meets the pad. The center section has a capacity of approximately 10 ml. The top section screws over this center section and, at its other end, is attached to rubber tubing leading to a pressure pump or to a positive-pressure nitrogen tank.

For sterilization, this small unit is attached to a vial with a rubber puncture cap, and has another small hypodermic needle stuck into the rubber cap to release any positive pressure.

A maximum of 10 pounds of pressure per square inch has been found to be ample in any filtration which we have attempted. A 10 ml. sample of serum will filter through steadily, drop by drop, without any care to the procedure,

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*Allergists Supply Company, 458 Broadway, New York 13, New York.

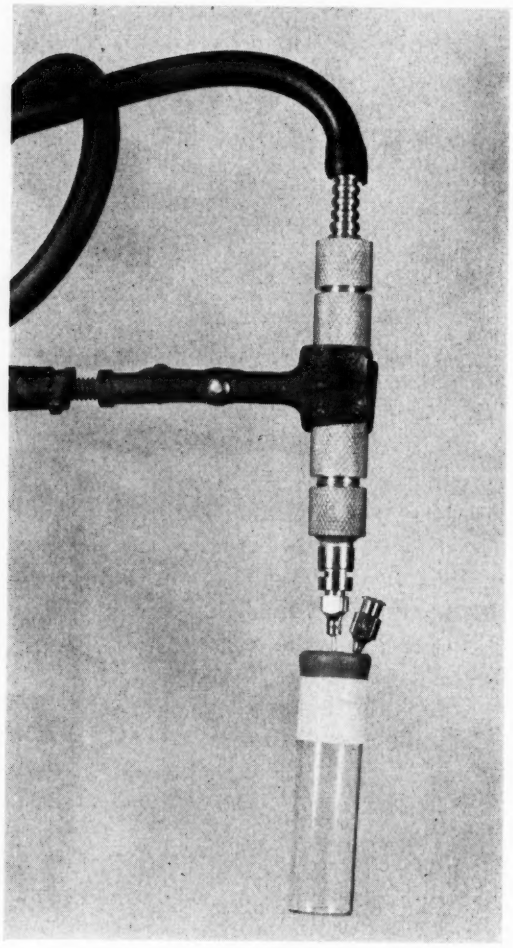

Fig. 1.

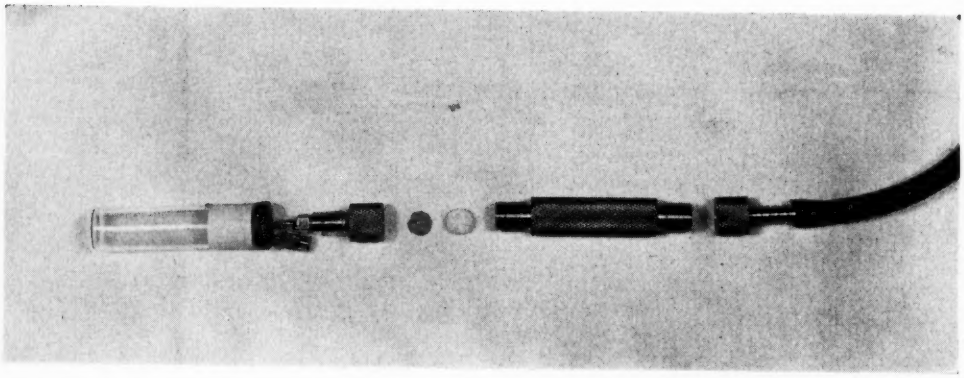

Fig. 2.

once it is set up. There is no loss of fluid through seepage. Should it be desirable to filter 20 to 30 ml. of fluid through this small filter to avoid adsorption of active material by a larger-surface filter, it is only necessary to remove the upper section of the filter, fill the center portion, replace the upper section, and continue the filtration.

So far, we have filtered several dozen sera and six different types of bacterial filtrates. All have had negative sterility tests after filtration through this unit.

REFERENCES

1. Allergists Supply Company: Allergy Circular, 458 Broadway, New York, N. Y.
2. Swinny, Boen: A Filter Adapter for Small Quantities of Solutions, J. Lab. & Clin. Med. **23**: 1098, 1938.

American Academy of Allergy

REPORT OF THE RESEARCH COUNCIL

THE Research Council held the first "plenary session" under its new Constitution and By-laws at the Hotel Statler in New York on Feb. 5, 1955. While it is too early to evaluate the effect of its administrative revision, the general impression was favorable and encouraging. The committees have been active. The reorganization has eliminated some inactive areas and has increased energy, ambition, and manpower for those projects which are within the scope of the Research Council. An earnest attempt has been made to expand the regional centers of study into a national network of joint effort.

The Committee on Aeroallergens (Secretary: Dr. Samuel M. Feinberg, 185 North Wabash Ave., Chicago 1, Illinois) is now divided into six subcommittees, as follows:

1. Mold and Pollen. (Secretary: Mr. Oren C. Durham, Abbott Laboratories, North Chicago, Illinois.)
2. Dust. (Secretary: Dr. Francis C. Lowell, 65 East Newton St., Boston, Massachusetts.)
3. Insect Allergens. (Secretary: Dr. Frank Perlman, 406 Medical-Dental Bldg., Portland 5, Oregon.)
4. Air Processing. (Secretary: Dr. Willard C. Small, 136 North Madison Ave., Pasadena 1, California.)
5. Weather (in process of organization).
6. Ragweed Control (in process of organization).

The Committee on Aeroallergens is the largest of the committees of the Research Council. Its secretary reported the following projects now in progress:

1. The Mold and Pollen Subcommittee is carrying on studies in about 100 cities in the United States, Canada, and Mexico. Dr. Ralph Voigt, Curator of the Academy Herbarium, has filled requests for 454 type specimens of pollens during the past year. Mold count studies have been made in a number of cities. Mr. Oren C. Durham's office gathered the data on pollen, while Dr. David Merksamer has been in charge of collecting data on molds. Dr. Merksamer's address (which has been inadvertently left out of the roster of the subcommittee in a recent issue of *News and Notes*) is 105 Lincoln Rd., Oakland 25, New York. This is important because letters concerning molds should be addressed to Dr. Merksamer, rather than to Mr. Durham. Both Mr. Durham and Dr. Merksamer have presented some of their data at the scientific exhibits at this meeting. Efforts to prevent spread of ragweed in western Oregon and central Washington are being watched. Among the other projects scheduled for 1955 are: observation of radial studies in pollen dispersal in New York State; trial of Millipore Filters for volumetric sampling; exhibit at the American Medical Association by O. C. Durham on "Ragweed Control—What Can and What Cannot Be Done."

2. The Subcommittee on Dust Allergy has made a survey of research on house dust, which is now carried out at various institutions throughout the United States. Current investigation of this subject appears to be proceeding along the following lines:

(a) Separation by chemical and physical means of the allergenically active component.

(b) Identification of the allergenic component in clinical and immunologic terms.
(c) Search for the source of the allergenic component.
(d) Identification and search for the source of other antigenic, but possibly non-allergic, components in house dust.

3. The Subcommittee on Insect Allergens has been active in initiating steps for investigation of the field. Dr. Perlman has interested an entomologist, Mr. Charles Woodworth, in cooperating. It is planned that the subcommittee will gather data on studies of insect allery and on the results of testing with insect allergens, act as a consulting group on problems in this field, and perhaps later provide allergens at a nominal cost. Dr. Perlman has already furnished to physicians a number of vials of insect allergens from his own supply. We cannot expect him, of course, to assume such a burden indefinitely.

4. Subcommittee on Air Processing. An experimental model of Raytheon's Micronaire filter was submitted to the Farr Company, 2031 East Rosecrans Ave., El Segundo, California, for test purposes.

A report of this test showed that this particular filter was highly efficient in its ability to remove small dust particles from the air. In fact, this method (the electrostatic precipitation method) was proved by this test to be far more efficient in the removal of pollen and small dust particles than any mechanical filter excepting those used in atomic energy plants, where the pressure drop across the filter is very great. Mr. Duncan, the chief test engineer for Farr, calculated the cost of doing the type of test as $175.00, which appears not unreasonable. This includes testing with various sizes of "standard dusts" and with ragweed pollen.

The Committee on Contactants (Secretary: Dr. Max Grolnick, 555 Ocean Ave., Brooklyn 26, N. Y.) was originally organized under the chairmanship of Dr. Marion B. Sulzberger to engage in "the study of specific problems concerned with the allergic responses of the skin to contact with various excitants." Its first joint effort was to be "a study for the assessments of procedures or techniques to determine the sensitizing potentials of unknown chemical substances." Inasmuch as such a venture would be costly and adequate funds are not available, the Committee on Contactants will dedicate itself to the following objectives:

(a) To act in an advisory capacity to individuals or groups seeking advice and guidance in problems of allergic responses of the skin to contact with various excitants.
(b) To formulate and recommend test programs in animals and man.
(c) To maintain liaison with Food and Drug Administration, U. S. Public Health Service, dermatologic and allergy societies, and other agencies interested in contact sensitivity.

The committee differs in its structure from the other committees of the Research Council. It has a large advisory council; its membership has been listed in the recently published roster of the Research Council.

Its function is to give advice, singly and collectively, in response to problems arising from requests by the laity, government agencies, industry, etc. In the past year a survey was carried out on the role of wool in railroad car upholstery as a possible source of allergic reactions.

Besides the advisory council, the committee consists of the following subcommittees:

1. Subcommittee on Testing Procedures
(Secretary: Dr. Rudolf A. Baer, 962 Park Avenue, New York 28, New York), which has two sections:

(a) A division of studies in animals
(Secretary: Dr. Merrill W. Chase, Rockefeller Institute, New York, N. Y.)
(b) A division of studies in man
(Secretary: Dr. Max Grolnick)

2. Subcommittee on Legislation

(Secretary: Dr. Naomi M. Kanof, 1150 Connecticut Ave., N.W., Washington 6, D. C.)

In accordance with the principles which govern the function of the Research Council, the remaining two subcommittees of the Committee on Contactants have been abolished: The Subcommittee on Miscellaneous Excitants and The Subcommittee on Miscellaneous Research. The secretary of the former Subcommittee on Miscellaneous Excitants, Dr. Morris Leider, 820 Caton Ave., Brooklyn 18, New York, has become a member of the council of the Committee on Contactants, with the provision that the Subcommittee on Miscellaneous Excitants might be reactivated at a future date. All subcommittees will act in an advisory capacity for the present. It is hoped that when grants in support of several of the projects now under the committee's consideration can be secured, it will then initiate an active investigative program of its own.

The Committee on Drugs (Secretary: Dr. Sidney Friedlaender, 10300 West Seven Mile Rd., Detroit 21, Michigan) does not have any subcommittee at this time. Dr. Carl E. Arbesman, who has guided its activities during the past seven years, has asked to be allowed to resign. Dr. Arbesman has been instrumental in introducing the rigid standards of basic research in the clinical evaluation of drugs. The secretary of the Committee on Drugs has submitted the following report:

> The Committee on Drugs of the Research Council of the American Academy of Allergy has been set up to study and to evaluate therapeutic agents which are claimed to be of benefit in the treatment of allergic disease. It does not supplant individual investigators, but rather enlarges on their observations and substantiates results by applying critical methods of scientific evaluation. The committee presently consists of eighteen fellows of the Academy practicing in various parts of the country. One of its members is Dr. Alex S. Friedlaender, whose name was unfortunately omitted from the last published roster of the Research Council. The services of the committee are offered without charge to reputable pharmaceutical manufacturers who wish to test the efficacy of a promising new agent, providing that adequate information concerning its background, pharmacology, and toxicity are supplied. All such preparations are first cleared with the pharmacologic consultants of the committee, Dr. Alfred Gilman and Dr. Carl Dragstedt. The results of such studies are presented both to the pharmaceutical house and to the members of the Academy. Another service performed by the committee is its ability to substantiate claims made for products or methods of treatment which are being given wide publicity in the medical or lay press. Members and fellows of the Academy are invited to submit problems or projects in which they are interested for consideration, as well as questions concerning types of treatment about which they have heard, and about which information is not readily available. A summary of all problems and questions submitted to the Committee, as well as the results of its investigations, are reported regularly to the Academy membership.
>
> At the present time the committee is evaluating a combination drug consisting of 25 mg. of Pyribenzamine and 5 mg. of Ritalin, submitted by the Ciba Company of Summit, N. J. Ritalin is a substance which has a stimulating activity somewhat between that of benzedrine and caffeine. Preliminary studies carried out during the past six months were reported at the February, 1955, meeting of the Academy. Evaluation will be continued during the coming pollen seasons. Several other projects are under consideration at this time, for investigation during the coming year.

The Committee on Food Allergy (Secretary: Dr. George I. Blumstein, 2039 Delancey St., Philadelphia 3, Pennsylvania) does not have any subcommittees, but its activities are divided into two separate, well-defined sections: (1) the maintenance of the reaginic bank, and (2) the investigation of the methods of preparing allergenic food extracts. Catherine

Bowman, B.A., is one of the most active members of the committee; we regret very much that her name was not included in the mimeographed roster of the committee which has been distributed in the spring of 1954 to the members of the American Academy of Allergy.

The secretary of the Committee on Food Allergy reports:

> (1) The reaginic serum bank has received several sera from other than local sources as the result of the publicity given it by the JOURNAL OF ALLERGY. We had only one request for processed serum. Knowledge of the existence of this bank and the availability of reaginic serum should receive greater publicity. It might be advisable to acquaint serologists and immunologists of this bank's existence by the insertion of small notices in journals most frequently read by them.
>
> Many inquiries have been received about the incidence of serum hepatitis and the methods of treating the sera to destroy the hepatotoxic agent. The reaginic bank has used ultraviolet irradiation by methods similar to those used by Dr. J. Stokes at his virus laboratory at the Children's Hospital in Philadelphia. Dr. Stokes' technique calls for a maximum of 10 c.c. of sera deposited in a 7 inch Petri dish and subjected to ultraviolet irradiation for fifteen minutes on a shelf that is kept in constant slow motion by an electric motor. This permits the irradiation of relatively thin layers of serum and, according to Stokes, destroys the virus. While the effectiveness of this method has been challenged by several recent publications, the group at the Brooklyn Jewish Hospital have done several thousand serum transfers without encountering a single case of serum hepatitis and, to avoid any possibility of medicolegal entanglements, your committee will continue to subject all sera to ultraviolet radiation.
>
> (2) The results of the questionnaire on the methods of preparing food extracts have been summarized and presented at the annual business meeting of the Academy on Feb. 8, 1955. The report will be forwarded to the editor of the JOURNAL OF ALLERGY, to be considered for publication. The plans of the committee call for a study on the allergenicity of food extracts prepared by different methods. The exact experimental procedure has not as yet been defined. It is likely that we shall deal with one variable at a time (for example, the effect of pH on the allergenicity of the resultant food extract, etc.)
>
> In 1955 the reaginic serum bank will be expanded by the establishment of collecting stations in the South, Midwest, and Pacific Coast. Satisfactory personnel is now being sought and will be supplied with sufficient forms and mailing cartons to encourage and expedite the acquisition of reaginic serum.
>
> We are ready to accept, and welcome, the names of volunteers who are willing to cooperate with us in this project.

The budget requested by the Research Council and approved for the current year is $2,600.00. Of this total, up to $600.00 has been earmarked for the administrative expenses of the office of the secretary; the monthly stipend, which the Executive Committee has granted the secretary of the Research Council in previous years, has been abolished. The remainder of the amount will be allocated to the committees, in accordance with their needs.

In recognition of the flexible demands of the committees, Dr. Bram Rose introduced the following motion at the plenary session, which was adopted by unanimous vote:

> It is suggested that the date of submission of the annual budget of the Research Council be changed from sixty days to three days prior to the annual meeting of the Academy, in order to allow discussion of needs by the Research Council, since the governing body of the Research Council meets only once during the year at this particular time.

The reports of the committees will be (1) presented at the plenary session of the Research Council, and (2) included in its annual report which is published in the JOURNAL OF ALLERGY.

In consideration of the suggestions of the Research Council, the Executive Committee has voted that in the future reports of individual committees of the Research Council shall

not be presented at the business meetings of the Academy; if formal reports, such as the reports of the Committee on Drugs or of the Committee on Food Allergy, seem to warrant wide distribution, they shall be submitted for consideration to the Program Committee of the annual meeting or to the editor of the JOURNAL OF ALLERGY.

In summary, the plenary session of the Research Council was a stimulating experience. It presented the highlights of an active year. It was full of promise. It came close to living up to a thought which was expressed by Dr. Blumstein at its closing—that this annual inventory of the activities of the Research Council should be more than a succession of reports or a discussion of budgetary and organizational problems; that it should be a "meeting of the minds."

Max Samter, M.D., Secretary.
University of Illinois College of Medicine
1853 West Polk St.
Chicago 12, Illinois

Announcements

Collegium Internationale Allergologicum

The Collegium Internationale Allergologicum held its second symposium in Basle, Switzerland, July 4 to 8, 1955. The subject of the symposium was "Migraine and Vascular Allergy." The program included well-known authorities from Western Europe and the United States. The proceedings will be published in the *International Archives of Allergy and Applied Immunology.*

Brazilian Currency Exchange

It has been suggested by Deak & Co., Inc., Foreign Exchange, that members going to Brazil will get better exchange in the United States than if they wait until arriving in Brazil. Deak & Co. may be reached in various cities, at the following addresses:

151 Maiden Lane, San Francisco, California
1406 New York Ave., N.W., Washington, D. C.
77 Adelaide St. West, Toronto, Canada.
Perera Offices, 10 Broadway, or 636 Fifth Ave., New York, New York.

Pollen Survey of Northern California

The Allergy Foundation of Northern California has been granted funds to carry out a field survey of hay fever-producing plants in northern California. This survey will be made by Professor McMinn of Mills College, Oakland, over a two-year period. The results of this survey will be published for general distribution.

Sociedad Mexicana de Alergistas

The ninth national annual meeting of the Sociedad Mexicana de Alergistas was held in the city of Leon, Guanajuata, from April 25 to 30, 1955. Twenty-four papers were presented at the scientific sessions.

An intensive course in allergic diseases was also conducted in the city of Leon from April 25 to 29, 1955, under the auspices of the Medical School of the University of Guanajuata by the Sociedad Mexicana de Alergistas in collaboration with the several local medical societies.

The new officers of the Sociedad Mexicana de Alergistas are: *President,* Dr. Jose Luis Cortes; *Vice-President,* Dr. Oscar de La Fuente; *Secretary,* Dr. Fernando Martinez C.; and *Treasurer,* Dr. Juan M. Lopez Sanabria.

In collaboration with the Academy's Committee on Aero-allergens, our Mexican Colleagues, under the leadership of Dr. Jose Luis Cortes, are making regular pollen counts at the following stations: Monterrey, Tampico, Durango, Oaxaca, Chiapas, and Mexico City.